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Susceptibility of Some Wheat (*Triticum aestivum* L.) Varieties to Aerosols of Oxidised and Reduced Nitrogen

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Abstract

In a pot culture (2011), aerosols of oxidized nitrogen ($NaNO_2$) @20 $kgha^{-1}$ yr^{-1} (≈200 ppm), reduced nitrogen (NH_4Cl) @10 kg^{-1} $hayr^{-1}$ (≈100 ppm) and distilled water (control) were sprayed (1500 cm^3 $plant^{-1}$ of each solution) weekly at different days after sowing (DAS) to study their impacts on physiology of wheat varieties (Ankur Omkar, Sonalika and K-306). In a field trial (2012), the simulated N-aerosols @ 200 ppm and 400 ppm (1000 cm^3 m^{-2} for each solution) along with a control were sprayed to population of the best variety (Ankur Omkar) at four different growth stages. In both pot and field experiments foliar feeding of aerosols with lower dose at the earlier growth stages influenced nitrogen use efficiency and yield. Fluctuations of cellular cations (Ca^{2+}, K^+) or peroxidase activity were caused by the aerosols and altered membrane permeability.

Keywords: Cations; Nitrogen use efficiency; Nitrate reductase; Net photosynthesis; Peroxidase; Membrane permeability

Abbreviations: $NaNO_2$: Sodium Nitrite; NH_4Cl: Ammonium Chloride; NUE: Nitrogen Use Efficiency; NR: Nitrite Reductase; CMS: Cell Membrane Stability; DAS: Days after sowing; CRI: Crown Root Initiation; P^n: Net Photosynthesis; d.w: Dry Weight

Introduction

Nitrogen oxides (NO, NO_2 and N_2O or NO_x) are recognized as atmospheric pollutants in rapidly growing urban and peri-urban areas of Northeast India [1]. Nitrogen dioxide gets dissolved in the extra-cellular water, form HNO_2 and HNO_3, which then dissociate to nitrate, nitrite and protons [2]. Reduced nitrogen incorporates into a wide range of compounds in nearly all higher plants [3]. Higher concentration (<10 μll^{-1}) of oxides of nitrogen alter the physiological processes including net photosynthesis and yields [4].

Reduced nitrogen (gaseous NH_3 or particulate NH_4^+ called NH_y) causes acidification of the ecosystem [5,6]. NHy contributes to a substantial portion of total deposition of nitrogen [7]. Ammonia at high concentration (>1 mM) is toxic to plants [8]. The consequences of high N deposition are seen in terrestrial ecosystem [9]. Foliar feeding of plants with aerosols of oxidised ($NaNO_2$) and reduced nitrogen (NH_4Cl) might affect growth stages and yield attributes of wheat varieties. We examined dose responses of wheat crop to N-aerosols and discussed mechanism(s) for varietal susceptibility or tolerance to the aerosols.

Materials and Methods

Cultural practices

Three wheat cultivars viz., K-306, Sonalika and Ankur Omkar were included in the pot experiments. A mixture (5 g) of urea, super phosphate and muriate of potash @2:1:1 were added to acid mineral soil in pots (size 5 kg) before raising plants from the seeds. In field, these fertilizers @ 80:46:42 per hectare were applied as basal before sowing of wheat seeds (variety: Ankur Omkar). The crop was irrigated during the growth periods to avoid desiccation. The experiments were laid in Randomized Block Design and replicated thrice.

Application of aerosols

In pot experiment 100 ppm NH_4Cl and 200 ppm $NaNO_2$ were sprayed on foliage at four growth stages viz., 0-30 DAS: germination cum crown root initiation (CRI); 30-60 DAS: tillering; 60-90 DAS: maximum tillering; and 90-120 DAS: reproductive development. Total volume of the each solution was 1500 cm^3 per stage. Similarly, solutions (1000 cm^3 per stage) each of 200 ppm NH_4Cl and 200 ppm $NaNO_2$ and distilled water (control) were sprayed to population of wheat crop at the four different growth stages. Drifting of the solutions from one pot or plot to another was prevented by using a hard board at the time of spray.

Measurement of net photosynthesis

We used transparent, airtight acrylic assimilation chambers (60 x 60 x 60 cm^3 for pot plants and 15 x 15 x 15 cm^3 field samples). Temperature (27-29°C), Relative humidity (52-57%) and Light intensity (23-27 Klux) inside the assimilation chambers were recorded by Hygrometer and Light meter respectively. The pot plants or leaf samples from field were incubated in presence of ambient 380 ppm CO_2 in sunlight during mid-day for half an hour. Inside air sample (10 cm^3) was collected by clinical syringe through the rubber port of the chambers, and injected into Environmental Gas Monitor (EGM-4) for measuring carbon dioxide concentration after incubation of plants inside the assimilation chamber. The rate of net photosynthesis was expressed as ppm CO_2 absorbed g^{-1} plant Dw h^{-1} [10].

NR activity in plants

Nitrate reductase activity was estimated based on conversion of nitrate to nitrite and inhibition of nitrite reduction to ammonia in anaerobic condition [11]. Green leaf samples (0.03 g) of 10-15 mm^2 size were put into 2.5 ml solution containing 200 mM phosphate

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buffer (pH 7.5), 30 mM KNO_3, 5% (v/v) propanol in assay tubes. The tubes were incubated in a water bath at 30°C for 30 minutes, at 100°C for 2 minutes and allowed them to cool to room temperature. To detect nitrite, 1 ml each of 1% sulfanilamide in 1N HCl and 0.02% N-(1-naphthyl)-ethylene diamine dihydrochloride were added to the solution. The solution was mixed thoroughly and kept in dark at room temperature for 15 min. Then, absorbency readings measured at 540 nm by spectrophotometer were plotted on a standard curve, which was prepared from a stock of 25 nmol nitrite per ml using KNO_2 in water.

Peroxidase activity in plants

Lipid peroxidation was measured in terms of Malondialdehyde content (MDA) [12]. Leaf sample (0.5 g) was homogenized in 10 ml of 0.1 per cent trichloroacetic acid. The homogenate was centrifused at 15,000 g for 5 minutes. The aliquot (2 ml) of the supernatant and 4 ml of 0.5% thiobarbuteric acid (TBA) in 20 per cent of TCA were mixed. The mixture was heated at 95°C for 30 minutes and cooled in ice bath. It was centrifused at 10,000 g for 5 minutes and the absorbance of supernatant was recorded at 532 nm. The value for non-specific absorption at 600 nm was subtracted from the value of 532 nm. The absorption coefficient of 155 nmol per cm was used to calculate MDA content as: MDA (nmol per g fresh weight) = (OD x 6)/0.155 x volume extract / (2 x weight of sample)

Nitrogen use efficiency (NUE) of crop

Kjeldhal method was used to determine total Nitrogen content, which is based on catalytic conversion of organic nitrogen into ammonia and its subsequent estimation by acid base titration [13]. NUE in grains of wheat varieties under treatments were calculated from their per cent Nitrogen and dry weight as: NUE% in grain = (Nitrogen % in grain x total grain yield per plant or per plot.

Measurement of CMS and estimation of cations

Cell Membrane permeability was measured in terms of CMS [14]. Twenty pieces of young leaves from the treated plants were cut into size of one centimeter square and immersed them first into 20 cm^3 distilled water in plastic bottles of 60 cm^3 capacity. The bottles were closed tightly to avoid leaking of the solution and checked gently using magnetic stirrer. Thus, freely water soluble ions in intercellular spaces of leaf were removed by the three serial washes with distilled water (each 10 min, 20 cm^3). Then, to extract the cations present in the exchangeable sites of the cellular locations, the same leaf discs were eluted by two treatments (each 1 h 20 cm^3) with 25 mM Sr_2Cl [15]. The solutions were collected into other plastic bottles. The plant samples were oven dried at 60°C to a constant weigh. The electrical conductivity readings of these solutions against the samples collected from the experimental treatments were used to compute CMS as follows.

$$CMS = [1-(1-T1/T2)/(1-C1/C2) \times 100]/ \text{Dry weight of leaf samples}$$

Where,

T_1= Conductivity reading of 25 mM Sr_2Cl (20 cm^3) without leaf samples

T_2= Conductivity readings of 25 mM Sr_2Cl (20 cm^3) with leaf samples

C_1= Conductivity reading of double distilled water without leaf samples

C_2= Conductivity readings of double distilled water with leaf samples

Intercellular and exchangeable Cations (K^+ and Ca^{2+}) extracted by distilled water and 25 mM Sr_2Cl respectively, were estimated using a Thermo Jarrel Ash S12 atomic absorption/emission spectrophotometer (Franklin, MA, USA) and air-acetylene mixture. Element contents of the leaves were expressed in terms of their oven dry weight.

Economic yield

Grains were threshed out from the plants under respective treatments at different growth stages of wheat crop and expressed as gram per plant (in case of pot plants) and quintal per hectare in field harvest.

Statistical analysis

Data were analysed following Generalised Linear Model (GLIM) program of Royal Society of London. Significant differences between two mean values due to treatments or varieties and their interaction at a crop growth stage were computed by comparing their significant levels at $P<0.05$.

Results

Net photosynthesis

In the pot experiment (Table 1), P^n at the germination-cum CRI stage of the crop (i.e.0-30DAS) varied significantly among the varieties. The variety Ankur Omkar had the highest P^n followed by Sonalika>K-306. In general, NH_4Cl lessened and $NaNO_2$ increased P^n by about 1% and 7.89% respectively. The P^n differed significantly due to treatments at tillering stage (i.e. 30-60 DAS) of the crop. There were 16.71% and 29.52% reductions of P^n NH_4Cl and $NaNO_2$ respectively. Variety Ankur Omkar had the highest P^n followed by K-306>Sonalika. Both varieties and treatments had significant effects on P^n at the maximum tillering stage i.e. 60-90 DAS. Variety K-306 showed the highest P^n

Varieties→Treatment ↓	Stage I (0-30DAS)		
	K-306	Sonalika	Ankur Omkar
Control	117.1	140.2	160.7
NH_4Cl (100 ppm)	151.7	124.1	138.8
$NaNO_2$ (200 ppm)	133.2	158.2	159.4
		SE diff(±)	LSD (0.05)
Variety		11.124	24.238
Treatment		-	n.s.
Variety x Treatment		-	n.s.

ppm CO_2 absorbed g^{-1} d.w. h^{-1}

Varieties→ Treatments↓	Stage II (30-60 days after sowing : DAS),			Stage III (60-90 DAS)			Stage IV (90-120DAS)		
	K-306	Sonalika	Ankur Omkar	K-306	Sonalika	Ankur Omkar	K-306	Sonalika	Ankur Omkar
Control	1064.9	830.3	744.2	899.6	1023.3	861.0	1262.8	748.8	678.3
NH_4Cl (100 ppm)	929.2	871.4	710.0	1046.8	662.0	730.9	537.2	629.7	861.7
$NaNO_2$ (200 ppm)	1103	877.9	729.0	691.9	691.9	623.2	796.9	668.3	613.7
SEdiff. (±)			LSD (0.05)	SEdiff. (±)		LSD (0.05)	SEdiff. (±)		LSD (0.05)
Variety		-	n.s.		91.72	164.17		151.98	331.18
Treatment		32.792	72.176		6518.7	14347		-	n.s.
Variety x Treatment	-	n.s.		0.358	0.810		-	n.s.	

SEdiff: Standard error of difference between two treatment means; Varieties or their interaction; LSD: Least significant difference; n.s: Non significant at P (0.05).

Table 1: Variation in rate of photosynthesis of wheat varieties following treatment at different days after sowing (DAS) in laboratory conditions. ppm CO_2 absorbed g^{-1} d.w. h^{-1}.

followed by Sonalika> Ankur Omkar at this stage. NH_4Cl and $NaNO_2$ brought down P^n by 9.89% and 15.704% respectively as compared to the control. The varieties had significant differences in respect of P^n at the reproductive development stage i.e. 90-120 DAS of the crop only. The order of P^n in varieties was K-306>AnkurOmkar>Sonalika.

In the field experiment (Figure 1), the treatments brought about significant changes in P^n of the wheat crop. Plants treated with the oxidised and reduced nitrogen had lower P^n by 5-6.5% only as compared to control. However, there were higher variations in P^n with respect to crop stages. The P^n declined at the later growth stages with application of the aerosols (Stage 1 (20.58%)>Stage 2 (46.82%)>Stage 3 (15.33%)>Stage 4). It may be due to the negative impact of the aerosols at the later growth stages as compared to the earlier ones.

Nitrate reductase activity activity

In the pot experiment (Table 2), both varieties and treatments had significant effects on NR activity of wheat crop. The effects of NH_4Cl on NR of varieties were negligible, whereas $NaNO_2$ increased NR activity merely (by 6.48%) as compared to control. Variety Ankur Omkar possessed the highest NR followed by Sonalika>K-306. At the maximum tillering stage, NR activity was significantly affected by variety and treatments. Variety Ankur Omkar had the highest NR followed by Sonalika > K-306. NH_4Cl enhanced NR activity by 34.07% and $NaNO_2$ by 7.69% as compared to control. Neither the variety nor the treatments at reproductive development stage exhibited significant changes in NR activity.

In the field experiment (Figure 2), NR activity varied significantly

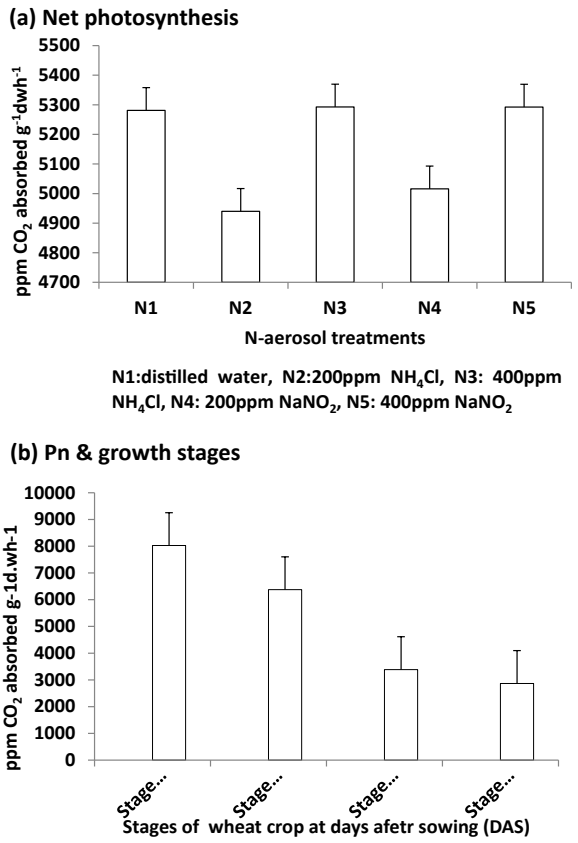

Figure 1: Net photosynthesis, pn & growth stages.

Varieties →Treatments ↓	Nitrate reductase activity at Stage I (0-30 DAS) (nm NO_3^- reduced g^{-1} h^{-1})		
	K-306	Sonalika	Ankur Omkar
Control	56.67	58.33	66.67
NH_4Cl (100ppm)	65.00	66.67	68.17
$NaNO_2$ (200 ppm)	68.33	65.00	68.33
	SEdiff ((±)	**LSD (0.05)**	
Variety	2.413	5.258	
Treatment	1.687	3.714	
Variety x Treatment	-	n.s.	

Nitrate reductase activity (nm NO_3^- reduced g^{-1} h^{-1})

Table 2: Variation in Nitrate reductase activity of wheat varieties following treatment at different days after sowing (DAS) in laboratory conditions.

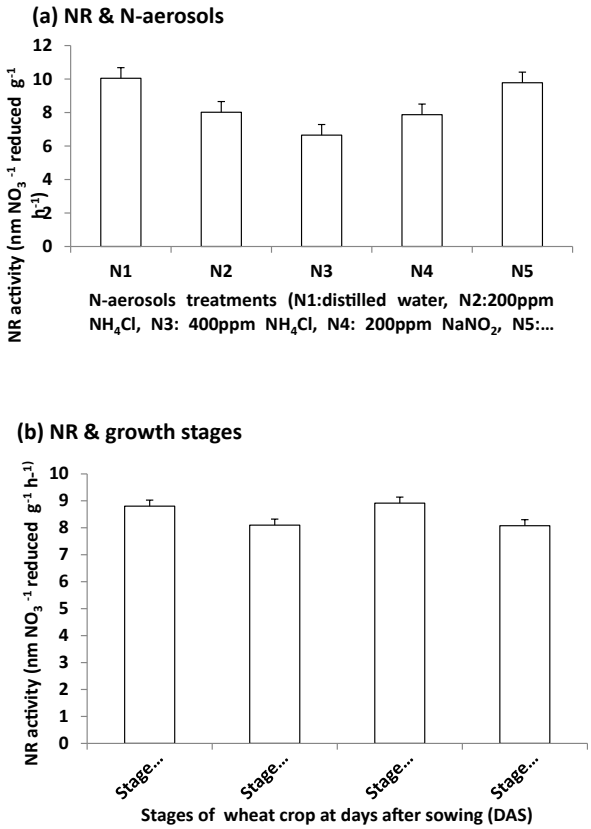

Figure 2: NR & N-aerosols, NR & growth stages.

due to the treatments with respect to time of application. Leaves treated with aerosols had lower NR activity. There were decreases in NR activity by $NaNO_2$ (21.62% and 28.0%) than by NH_4Cl (19.39%, 11.26%) at 200 and 400 ppm respectively. Also, lower NR activity was found with the higher doses of the aerosols.

Nitrogen use efficiency

Data on NUE in wheat grains from the pot experiment are presented in Table 3. In this experiment, there were 1.87% and 8.88% reductions of grain NUE in NH_4Cl and $NaNO_2$ treated plants respectively. Some differences in NUE were found among the varieties, where Sonalika>Ankur Omkar>K-306. The variety and treatments had significant effects on grain NUE at maximum tillering stage of the crop.

Varieties →Treatments ↓	Stage II (30-60 DAS)			Stage III (60-90 DAS)			Stage IV (90-120 DAS)		
	K-306	Sonalika	Ankur Omkar	K-306	Sonalika	Ankur Omkar	K-306	Sonalika	Ankur Omkar
Control	10.00	12.50	18.33	21.67	36.67	25.00	38.75	53.33	50.84
NH_4Cl (100ppm)	8.33	16.67	18.33	28.33	66.67	31.67	53.34	47.50	52.00
$NaNO_2$ (200 ppm)	33.33	13.33	12.50	50.00	48.33	51.67	55.33	41.67	65.83
		SEdiff (±)	**LSD (0.05)**		**SEdiff (±)**	**LSD (0.05)**		**SEdiff (±)**	**LSD (0.05)**
Variety		-	n.s.		9.274	20.207		-	n.s.
Treatment		-	n.s.		6.867	15.115		-	n.s.
Variety x Treatment		-	-		-	n.s.		-	n.s.

SE diff: Standard error of difference between two treatment means, varieties or their interaction; LSD: Least significant difference; n.s: Non significant at P (0.05).

Table 3: Variation in Nitrogen use efficiency (NUE) in grain of wheat varieties following treatment at different growth stages (Days after sowing: DAS) in laboratory conditions.

There were 16.1% and 19.1% increases in grain NUE by NH_4Cl and $NaNO_2$ respectively. Variety Ankur Omkar ranked first followed by Sonalika > K-306 in case of grain NUE. The N aerosols had significant effects on the grain NUE at reproductive development stage of the crop. There were 21.99% and 27.03% increases in grain NUE by NH_4Cl and $NaNO_2$ respectively. The variety Ankur Omkar had higher grain NUE than K306>Sonalka varieties.

In the field experiment, NUE (Figure 3) increased in leaf at 400 ppm NH_4Cl as well as 200 ppm and 400 ppm $NaNO_2$ treatments (by 25.47%, 26.17% and 4.38% respectively). NUE fluctuates at different days after sowing (Stage 2 (18.84) % >Stage 4(17.27%)>Stage 3 (9.68%)>Stage 1).

Cell membrane stability

In the pot experiment (Table 4), CMS was affected significantly by the treatments at tillering stage of wheat crop. Variety Ankur Omkar>K-306>Sonalika had differences in CMS. Both varieties and treatments had significant effects on CMS of wheat crop at maximum tillering stage. K-306>Ankur Omkar>Sonalika had a variable CMS. On an average, N-aerosols applied at the reproductive development stage, had significant effects on CMS of Wheat crop. Ammonium chloride and Sodium nitrite increased CMS up-to 46.62% and 84.46% respectively as compared to control. The varieties Sonalika>Ankur Omkar>K-306 had some differences in their CMS.

In the field experiment (Figure 4), Cell membrane stability of wheat did not differ significantly for the treatments. However, a higher CMS was found in early application of the aerosols than at the later stage (Stage 1>Stage 2>Stage 4>Stage 3 by 1.22%, 13.63%, 5.36% respectively).

Economic yield

In the pot experiment, economic yield of wheat varieties corresponding to the treatments are presented in Table 5. N-aerosol applied at tillering stage of the crop had significant effects on grain yield. NH_4Cl decreased (by 14.95%) and Sodium nitrite increased (by 21.74%) of grain yield. The varieties K-306>Ankur Omkar>Sonalika had variations in their grain yield. The treatments at the maximum tillering stage had significant effects on economic yield. NH_4Cl and $NaNO_2$ increased grain yield by 22.9% and 42.81% respectively at this stage. Varieties Ankur>K-306>Sonalika differed from each other in grain yield production. The application of N-aerosols at the reproductive development stage couldn't change grain yield significantly.

In field experiment, the treatments had significant effects on economic yield of wheat crop (Figure 5). In general, NH_4Cl could not change but $NaNO_2$ decreased economic yield by 15.03% and 25.81% at 200 ppm and 400 ppm respectively. Lower was the concentration of the oxidised aerosol, higher was the production of yield of the crop. There were reductions in yield with application of aerosols at later stage (Stage1>Stage4>Stage3>Stage2).

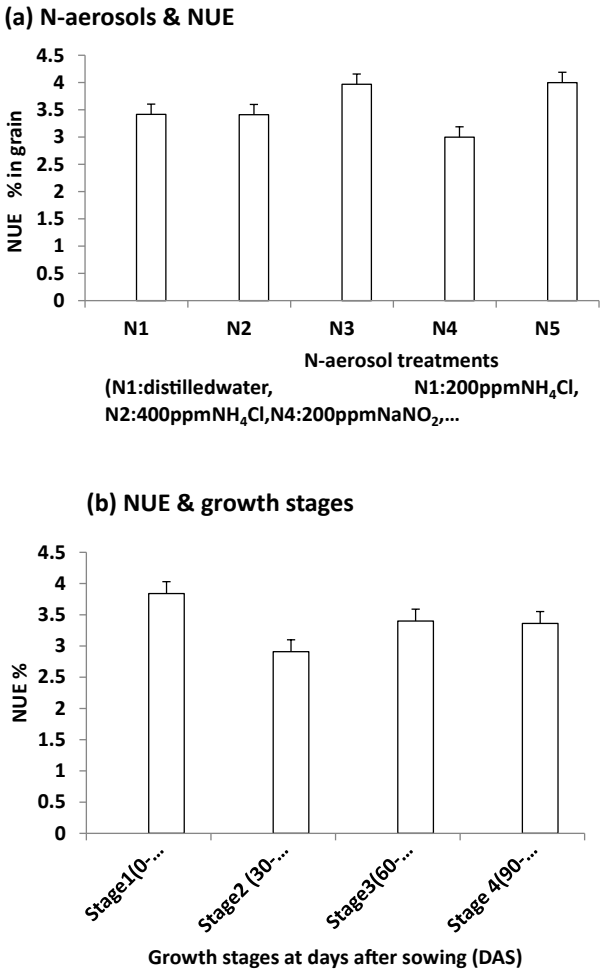

Figure 3: N-aerosols & NUE, NUE & growth stages.

	CMS (index)								
Stages →	**Stage II (30-60 DAS)**			**Stage III (60-90DAS)**			**Stage IV (90-120 DAS)**		
Varieties→ Treatment-s ↓	K-306	Sonalika	Ankur Omkar	K-306	Sonalika	Ankur Omkar	K-306	Sonalika	Ankur Omkar
Control	-1.34	-0.82	0.89	20.34	9.70	8.71	1.390	6.99	10.33
NH_4Cl (100 ppm)	6.32	3.60	4.56	7.420	12.79	8.31	7.77	4.95	22.36
$NaNO_2$ (200 ppm)	5.48	3.09	5.64	33.62	9.65	17.52	29.36	56.46	34.59
		SEdiff (±)	**LSD (0.05)**		**SEdiff (±)**	**LSD (0.05)**		**SEdiff (±)**	**LSD (0.05)**
Variety		0.687	1.512		4.499	9.902		6.307	13.88
Treatment		-	-		5.802	12.643		-	n.s.
Variety x Treatment		-	n.s.		-	n.s.		-	n.s.

SE diff: Standard error of difference between two treatment means, varieties or their interaction; LSD: Least significant difference; n.s: Non significant at P (0.05). Data on first stage are not available due to plant mortality.

Table 4: Cell membrane stability (CMS) of wheat varieties following treatment at different growth stages (Days after sowing: DAS) in laboratory conditions.

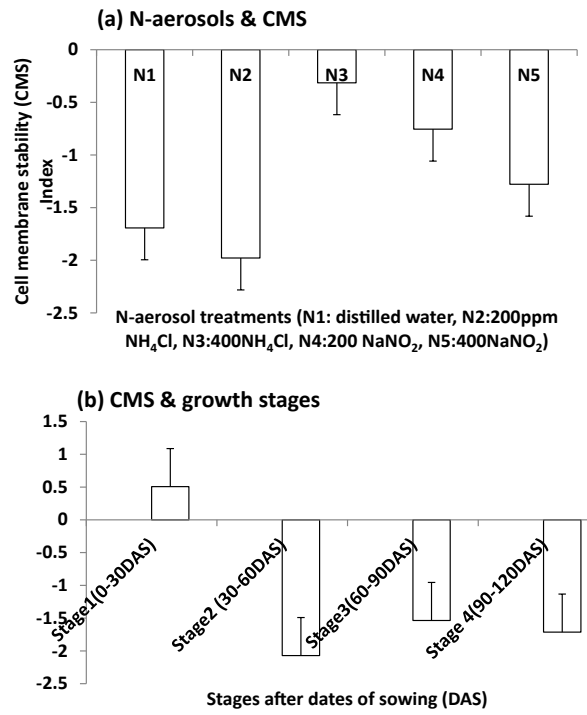

Figure 4: N-aerosols & CMS, CMS & growth stages.

Varieties → Treatments ↓	Economic Yield (g plant^{-1})								
	Stage II (30-60 DAS)			Stage III (60-90 DAS)			Stage IV (90-120 DAS)		
	K-306	Sonalika	Ankur Omkar	K-306	Sonalika	Ankur Omkar	K-306	Sonalika	Ankur Omkar
Control	19.143	21.620	36.34	17.73	18.18	29.68	23.24	21.96	23.72
NH_4Cl (100 ppm)	20.743	9. 720	20.01	14.75	18.60	28.47	26.34	19.51	24.93
$NaNO_2$ (200 ppm)	19.519	16.774	22.30	15.48	26.72	26.17	28.87	248.78	28.45
		SEdiff (±)	LSD (0.05)		SEdiff (±)	LSD (0.05)		SEdiff (±)	LSD (0.05)
Variety		5.535	12.183		5.131	11.293		-	n.s.
Treatment		-	n.s.		-	n.s.		-	n.s
Variety x Treatment		-	n.s.		-	n.s.		-	n.s.

SE diff: Standard error of difference between two treatment means, varieties or their interaction; LSD: Least significant difference; n.s: Non significant at P (0.05). Data on first stage are not available due to plant mortality.

Table 5: Variation in economic yield of wheat varieties following treatment at different days after sowing (DAS) in laboratory conditions.

Distribution of cations

Cations (Ca^{2+} and K^{+}) present in intercellular and exchangeable sites varied significantly among the wheat varieties due to the aerosol treatments (Table 6). NH_4Cl depleted more cations from the water free spaces and exchangeable sites than the oxidized nitrogen.

Peroxidase activity

The PO activity (Figure 6) in leaf of wheat (variety: Ankur Omkar) was determined in the field trial only. There were significant effects of the nitrogenous aerosols on PO at different DAS. The leaves treated with aerosol of oxidised nitrogen had higher PO (by 16.56% and 13.57%) than the reduced nitrogen (2.259% and - 1.83%) at 200 and 400 ppm respectively. The PO also increased commensuration with crop growth stages (Stage1 (37.03%)< Stage2(4.82%)< Stage 3 (65.74%)<S4).

Discussion

The present investigation into the impacts of oxidized and reduced nitrogen aerosols (viz., $NaNO_2$ and NH_4Cl respectively), applied at different growth stages of Wheat (*Tritcum aestimum* L.) crop, deals with physiological parameters contributing to higher grain yield production. We also attempted to sort out the most suitable variety and its stage responding positively to the aerosol of nitrogen. The mechanism(s) of injury to the varieties by the nitrogenous compounds are discussed in this paper [16].

In pot culture experiment, the simulated aerosols were NH_4Cl @100 ppm (≅10 kgNha^{-1} yr^{-1}) and $NaNO_2$ @ 200 ppm (≅20 kgNha^{-1} yr^{-1}) respectively. These were applied at different growth stages of wheat crop. We preferred the N-aerosols as foliar feeding to fumigating plants. The rate of P^n in wheat varieties varied significantly following foliar feeding. The P^n rate in crop varieties also different due to the differences in nitrogen input from the aerosols at their growth stages. There are several previous reports on P^n depression in higher plants by nitrogenous pollutants. A concentration at 0.6 µll^{-1} may cause P^n inhibition of oat (*Avena sativa* L.) and alfalfa (*Medicago sativa* L.) during 90 min fumigation [17]. Similarly there is evidence that decrease in P^n of bean (*Phaseolus vulgaris*) is related to 1-7 µll^{-1} NO_2 over a period of 5 h [18]. Chloroplastic pH increases when the number of protons in the chloroplast exceeds the proton required (six) for a NO_2 reduction. The key enzyme of carbon assimilation (ribulose-1-5-bisphosphate carboxylase/oxygenase) is pH dependent. Such changes in pH are likely to be harmful [19]. Photosynthetic depression has also been related to ultra structural changes in plants by NO_2. The changes include protrusion from the chloroplast [20] and swelling of thylakoids [21]. Sodium and chloride ions might enter into cells, but they were

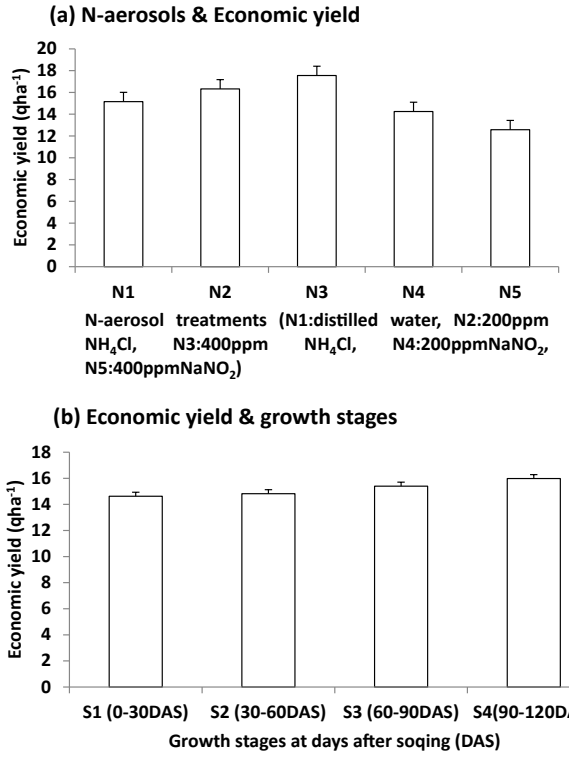

Figure 5: N-aerosols % Economic yield.

Varieties (V:1,2,3)→	K-306 (mMg^{-1}d.w.)			Sonalika (mM g^{-1}d.w.)			Ankur Omkar (mM g^{-1}d.w.)		
Treatments (T:1,2,3) →	DistilledH_2O (0ppm)	NH_4Cl (100 ppm)	$NaNO_2$ (200 ppm)	DistilledH_2O (0 ppm)	NH_4Cl (100 ppm)	$NaNO_2$ (200 ppm)	DistilledH_2O (0ppm)	NH_4Cl (200 ppm)	$NaNO_2$ (200 ppm)
30-60 DAS									
Intercellular Ca^{2+}	19.86	26.80	29.11	19.36	27.42	28.51	15.59	22.80	25.61
at P(0.05) T1,T2<T3 &V3<V1,V2 only									
Exchangeable Ca^{2+}	55.71	27.29	65.70	51.62	44.63	65.94	54.92	45.61	51.87
Intercellular K^+	1.12	1.10	1.64	1.18	1.61	2.02	0.97	1.57	2.09
at P(0.05) V1<V2 only.									
Excahgeable K^+	2.88	1.70	3.28	2.44	2.54	3.66	2.25	2.64	2.91
at P(0.05) V2<V1<V3									
60-90 DAS									
Intercellular Ca^{2+}	18.33	12.92	19.97	39.19	17.82	13.52	32.02	39.77	27.36
at P(0.05) V1<V2,V3 only									
Exchangeable Ca^{2+}	24.47	23.60	26.85	43.11	26.73	17.88	34.48	109.07	34.38
Intercellular K^+	1.39	1.11	1.53	1.47	0.75	0.57	1.87	2.56	2.07
at P(0.05) V2<V1<V2 only									
Excahgeable K^+	2.42	2.86	1.91	3.15	1.94	2.86	3.10	4.71	2.50
at P(0.05) V1<V2<V3 only									
90-120 DAS									
Intercellular Ca^{2+}	4.43	88.87	59.78	37.77	67.08	150.34	71.82	51.82	124.20
at P(0.05) T1<T3 only									
Exchangeable Ca^{2+}	16.45	114.02	215.71	99.45	121.22	255.90	107.73	97.79	195.96
at P(0.05) T1<T2, T3 &V1<V2 only									
Intercellular K^+	0.32	2.65	4.09	4.33	4.10	4.38	5.01	14.83	8.15
at P(0.05) T<T2 only									
Excahgeable K^+	0.77	6.19	9.92	4.05	6.08	19.42	5.18	8.63	10.53
at P(0.05) T1<T2<T3 only									

P (0.05): Significant at 5% probabability level.

Table 6: Effects of oxidised ($NaNO_2$) reduced (NH_4Cl) nitrogen aerosols on distribution of Calcium and Potassium ions in intercellular and exchangeable sites of Wheat (*Triticum aestivum* L.) crop treated at different days after sowing (DAS) under laboratory conditions.

neglected as non-physiological at relatively alkaline pH (>5.0) of the aerosols in our work.

The NR Nitrate activity in wheat was lowered in shoots by NH_4Cl and $NaNO_2$ at almost all stages of the crop. The varieties differed in respect of NR in their leaves. Nitrate reductase catalyses the reduction of nitrate to nitrite, and its levels of activity are determined by the supply of nitrate [22]. Inhibition of NR activity may be crop specific also [23]. The accumulation of larger amount of ammonium ions and certain amino acids in squash cotyledons during fumigation reduces NR activity [24].

In the present study, NUE in wheat increased with treatments of N-aerosols at their growth stages except a few. On an average, varieties also showed remarkable differences in their NUE in grains. However, NUE in grains decreased with the higher concentration of nitrogen aerosols in both pot culture and in field experiments. The differences between accessions in the response to N for physiological and phonological variables exists in case of Arabidopsis lyrata petrae [25]. Moreover we found that nitrogen nutrition enhanced grain yield of wheat varieties irrespective of their growth stages. The enhancement is more prominent in $NaNO_2$ than NH_4Cl fed plants. Higher nitrogen use efficiency with lower quantity of nitrogen from the source might improve nutritive quality of the crop varieties [26] but it showed a negative impact on productivity of cereal crops. The wheat varieties having higher grain yield were found with treatments at maximum tiller formation stage (60-90 DAS). In wheat, Ankur Omkar followed by K-306 and Sonalika emerged as commendable varieties. In these potential varieties, cell membrane stability was found to be higher irrespective of treatments. Cell membrane permeability was increased (with lower CMS) by both NH_4Cl and $NaNO_2$ as compared to control. As NH_4Cl treated plants had higher leakage of ions from the cells, they possessed higher quantum of the cations in the intercellular and exchangeable sites. Similarly, $NaNO_2$ treated plants had higher CMS and lower membrane leakage than NH_4Cl treated plants, a lower amount of the cations were recovered from the cellular locations. The rate of PO activity of wheat crop treated with $NaNO_2$ was higher than the rate shown by the NH_4Cl treatment as compared to the control. Therefore, the membrane damages caused by NH_4Cl and $NaNO_2$ were brought by two different mechanisms. The former depleted the cations from the membrane directly and the later caused peroxidation of lipids present in the membrane. Hence, the membrane became leaky for the cations, and their quantum was higher in the intercellular and exchangeable sites irrespective of varieties, which were detected in the extraction processes with water and $SrCl_2$ solutions respectively. Although, nitrite causes swelling of thylakoids and changes membrane stability, direct interference of free radicals with critical enzymes [27] may be responsible for reduction in growth and yield of crops. The oxides of nitrogen following the lipid breakdown in membrane cause cellular plasmolysis [28]. Apart from uncoupling electron transport chain in chloroplast [29], ammonia reduces cations viz., Calcium, magnesium, and potassium [30]. In plant cells, calcium is one of the integral components of plasma membrane, where it helps maintain stability [31]. Calcium ions binds with modulator proteins e.g. calmodulin [32], and serves as chemical signaling that in some cases

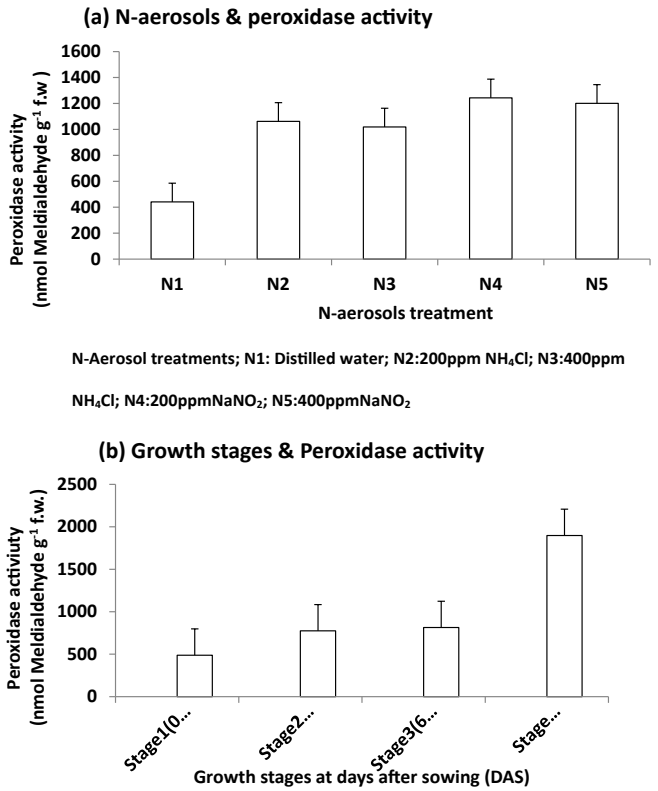

Figure 6: N-aerosols & peroxidise activity.

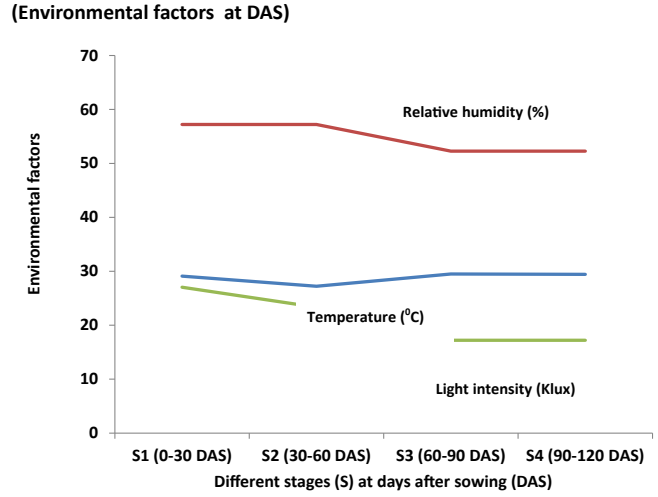

Figure 7: Environmental factors at DAS.

equips the plant to resist external stresses [33]. These possibilities have not been explored meticulously in the present studies.

In the study, data were reproduced from pot and fields experiments in natural environmental conditions. The plants faced with varying in light intensity, temperature, relative humidity during their growth periods, and particularly during incubation period for measurement of net photosynthesis. It was clear from presentation of environmental data that none but the light intensity varied mostly (Figure 7). Light might have some roles on physiological variations and productivity of the crop stage-wise. There are differences between species imparting carbon dioxide fixation, and reduction of nitrite only at low light levels and high nitrite concentration [34]. So, the effects of irradiance, temperature and even desiccation on the pollution responses of the selected crop varieties and role of oxidative damage in these responses are important thrusts. All these largely indicate that the changes of the physiological parameters and productivity of the winter wheat crop may have quite different facets in context with the impacts of nitrogenous pollutants.

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