Effects of Nitrogen Aerosols on Biochemical Traits of Some *Kharif* Rice (*Oryza sativa* L.) Genotypes

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Abstract— *Nitrogen (N) is a vital plant nutrient for crop growth and development. In tropics and subtropics, nitrogen is lost by leaching, runoff and volatilization. Foliar nutrition in crops has increased in importance alternative to soil application. In nature, the input of nitrogen is possible through dry and wet deposition of nitrogen or as aerosols on foliage. The responses of rice crops to aerosols of nitrogen linked to its productivity deserve strong attention in the present climate change scenarios globally. Therefore, a field experiment (2020) was conducted in ICR Farm, Assam Agriculture University to investigate into effects of N-aerosols on biochemical traits in 10 (ten) kharif rice genotypes. The aerosols viz., KNO_3: NH_4NO_3 and $Ca(NO_3)_2$ @60 kg ha^{-1} (≈600ppm), 1000 cm^3 each were sprayed at maximum tillering and heading stages of the rice crop. In the experiment, the nitrogen aerosols increased biochemical traits viz., contents of chlorophyll 'a' (40.91-91.66%) & (10.52-47.22%), chlorophyll 'b' (4.91-19.67%) & (6.89-10.34%), total chlorophyll (21.51-53.96%) & (23.42-39.03%), NR activity (64.21-95.92%) & (50.66-72.39%) at maximum and heading stages respectively, nitrogen in grains (13.04-22.61%), and protein in grains (13.49-20.72%). Moreover, there were increments in lipid peroxidation (4.71-107.14%) and Cell membrane stability (CMS) (5.72-29.85.92%), intercellular [K^+] (20.23-70%), exchangeable [K^+] (68.46-77.02%), intercellular [Ca^{2+}] (6.77-17.80%) and exchangeable [Ca^{2+}] (3.29-33.80%), which varied significantly depending on the types of the N-aerosols. In the experiment, the variety Gitesh showed the highest response to the N-aerosols irrespective of the crop growth stages. Further, among the N-aerosols, KNO_3 was found to be the best followed by $Ca(NO_3)_2$ & NH_4NO_3, control distilled water in terms of the traits.*

Keywords— Aerosols, Chlorophyll, CMS, Nitrogen, NR, Protein.

I. INTRODUCTION

Nitrogen is indispensable for plant growth, as it makes up 1 to 4% of the dry matter of the plants. In proteins and nucleic acids, it is a major component. It acts as an essential constituent of chlorophyll and many other enzymes (Leghari *et al*., 2016). Therefore, the availability of nitrogen in sufficient quantity throughout the growing season is essential for optimum growth and production of crop plant. There is also importance of nitrogen to maintain its correct ratio (12:1 to 15:1) with sulphur in improving yield and quality of crops (Klikocka and Marks, 2018). In order to increase crop production, approximately 107.55 million metric tons of N fertilizers were applied globally in 2019 (Statista, 2022). Approx. 80 million tones of the total N fertilizer is utilized for cereals globally (FAO, 2014). Generally, only 40–50% of the applied N fertilizer is utilized by the crop (Sylvester-Bradley and Kindred, 2009). Leaching, runoff, volatilization, and denitrification are major causes of nitrogen losses in the environment. Nitrogen which is lost from the plant–soil system can result in environmental problems, including water and air pollution (Sainju, 2017). Dissolved N in ground water mostly in the form of NO_3^{-1} and $NH_4^+$are leached out or lost through gaseous emissions of nitrous oxide and ammonia (Neill *et al*., 2005). Janzen and Ellert (1998) observed that nitrogen is lost by the denitrification mechanism from agricultural soils. Neill *et al*. (2005) reported a loss of 20–40% of the N applied in the soil. In general, Urea or Diammonium phosphate (DAP) is used at basal and maximum tillering stages of rice crop, which is prone to the environmental losses. Apart from these forms of synthetic N, knowledge on fertilizing crops with N-aerosols is lacking. Because, N may be received from the atmosphere as a consequence of lightening or dry and wet deposition of N on

foliage at physiological pH. In this context, the responses of *kharif* rice genotypes to nitrate and ammonium forms of nitrogen aerosols are poorly understood.

A stable suspension of solid and liquid particles in a gas is known as aerosols. Aerosol particle size varies in between 0.001 to 100µm (Colbeck and Lazaridis, 2010). Nitrogen oxides (NO_X) *viz*., NO and NO_2, and N_2O are major atmospheric pollutants in rapidly growing urban and its surrounding areas of Assam (Bharali *et al.*, 2012). The NOx emitted in the form of nitric oxide (NO), reacts rapidly in the atmosphere and in a complex cycle with light, ozone, hydrocarbons, and produces nitric acid. These materials interact with plants and soil locally or transported from the site to react with atmospheric particulates to form aerosols. These aerosols come back to fertilize terrestrial and aquatic systems in the form of wet and dry deposition. It was estimated that about 5% of the total anthropogenic greenhouse effect is credited to N_2O form, which is 70% of annual global anthropogenic emissions come from animals and crop production (Arvind, 2001). There is the possibility of nitrogen nutrition in rice by foliar feeding with N-aerosols (salts of nitrogen) instead of soil application (Bharali et al., 2017). Therefore, an investigation into effects of N-aerosols on biochemical traits of rice crop especially NUE in rice crop was undertaken.

II. MATERIALS AND METHODS

A field experiment following factorial randomised design with two replications was conducted to study the biochemical variations of ten *kharif* rice genotypes (viz., Aghonibora, Gitesh, Aki Sali, Prafulla, Solpana, Bahadur, Ranjit, Swarna sub-I, Chahou, Shruboni) upon application of nitrogen aerosols (KNO_3 , NH_4NO_3 & $Ca(NO_3)_2$ each @ 60 kg ha^{-1} against a control i.e. distilled water) during *kharif* season (July -December) of 2020 at ICR Farm (GPS at 26°45'N latitude and 94°12'E longitude) under Assam Agricultural University, Jorhat. The climatic condition during the experimental period was characterised by Temperature (10.5-33.6^{0}C), Relative Humidity (61-99%), total bright sunshine (28hours) and total rainfall: 1110.2mm (Source: Meteorological observatory, AAU, Jorhjat). There were variations in canopy temperature (measured by Infrared Thermometer at an angle of 45° and at a distance of 10 cm from the leaf) among the varieties at maximum tillering (19.95-22.79^{o}C) and heading (20.06-23.13^{o}C) stages of the crop irrespective of treatments.

The aerosols @ 600 ppm (≈60 kg N ha^{-1}), solution was prepared by dissolving 600mg each of KNO_3 , $Ca(NO_3)_2$ and NH_4NO_3 in small amount of distilled water separately, and then volume was made upto 1000ml by distilled water. These nitrogen aerosols were applied in three splits on sunny days in the afternoon (after 2–3 p.m) when air temperature was low. Spraying of nitrogen aerosols was finished in such a way that each variety obtained 1000cm^3 of the respective nitrogen aerosols solutions. Treatments were applied at 30 days after transplanting at maximum tillering stage of the crop as foliar misting using knapsack sprayer. The pHs of the respective solutions measured using a digital pH meter (Eutech pH 510) were as for distilled water: 7.00; KNO_3: 5.66; $Ca(NO_3)_2$: 5.49 and $NH_4(NO_3)$: 5.89. The estimation of biochemical indices were done as per the standard methods as mentioned below:

Sl.No.	Methodologies	References
1.	Clorophyll (a, b & total) contents (mgg^{-1}fresh weight of leaf)	Colorometric method (Arnon, 1949)
2.	Nitrate Reductase (NR) activity (µmoles NO_2 formed g^{-1}fresh tissue wt hr^{-1}) in plants	Keeper *et al.* (1971)
3.	Lipid peroxidation (n mol MDA g^{-1} fw)	Heath and Packer (1968)
4.	Cell membrane stability (CMS)	Sullivan (1979), Bharali *et al*. (2015)
5.	Estimation of cellular K^+	Jackson (1973)
6.	Estimation of cellular Ca^{2+}	Ca^{2+} (Richards, 1954; Bharali *et al*., 2015b)
7.	Nitrogen and protein contents in grains (%)	Jackson (1973)

Data for each character was analysed by Fisher's method of analysis of variance (Panse and Sukhatme, 1978). 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 $P<0.05$.

III. RESULTS AND DISCUSSION

In the study, simulated N-aerosols viz., KNO_3, $CaNO_3$ and NH_4NO_3 @ 600 ppm each (≈60 kg N ha^{-1}) along with a control, applied at different growth stages of winter rice crop, brought about significant biochemical changes in the crop. It was aimed to find out the most responsive variety corresponding to the type of N-aerosol judged by the biochemical traits at different growth stages of the crop, as explored in the discussion.

In the experiment, during maximum tillering stage (Table 1a), the highest increment in chlorophyll 'a' content was found in treatment $Ca(NO_3)_2$ (91.66%) > KNO_3 (83.33%), and the lowest was in NH_4NO_3 (40.91%) as compared to the control. During heading stage (Table 1b), the highest per cent increase in chlorophyll 'a'content was found in treatment KNO_3 (47.22%) > $Ca(NO_3)_2$ (42.11%), and the lowest was in NH_4NO_3 (10.52%) as compared to the control.

Plant pigments viz., chlorophylls are vital molecules that are formed by plants and plays an important role in photosynthesis process which is accountable for plant growth (Brotosudarmo *et al.*, 2018). Qeyami *et al.* (2020) reported that application of calcium nitrate enhanced the nitrogen content in leaves and greater absorption of Mg^{2+} from soil, and thus attributed to raised chlorophyll 'a'content in leaves of apple plant. Zhao *et al.* (2016) stated that in maize both Chl a and Chl b got reduced under KNO_3 deficiency conditions because potassium deficiency directly influence the photosynthetic apparatus through biosynthesis and functioning of key photosynthetic components whereas nitrogen deficiency reduces the photosynthetic enzymes and chlorophyll components.

In the experiment, during maximum tillering stage (Table 2a), the highest increment in chlorophyll 'b' content was found in treatment $Ca(NO_3)_2$ (19.67%) > KNO_3 (18.03%), and the lowest was in NH_4NO_3(4.91%) as compared to the control. During heading stage (Table 2b), the highest increment in chlorophyll 'b' content was recorded in treatment KNO_3 (10.34%) > $Ca(NO_3)_2$ (8.78%), and the lowest was in NH_4NO_3(6.89%) as compared to the control. Chlorophyll b is different from Chlorophyll a in only one of the functional groups bonded to the porphyrin ring (Xu *et al*., 2001). Chlorophyll b is an accessory pigment and acts indirectly in photosynthesis by transferring light energy to Chl a (Eggink *et al*., 2001). El- Mogy *et al.* (2019) proposed that potassium nitrate application in long pepper plant improved the stability of protein complex and chloroplast structure and thus maintained the Chl a and Chl b contents. Norozi *et al.* (2019) illustrated that potassium nitrate application caused significant improvement in Chl a and Chl b because of potassium regulates various enzymes related to photosynthesis, and further it assist in detoxification of ROS which prevent the degradation of chlorophyll contents.

In case of total chlorophyll contents, at maximum tillering stage (Table 3a), the highest per cent increase in total chlorophyll content was found in treatment KNO_3 (53.96%) > $Ca(NO_3)_2$ (43.79%), and the lowest was in NH_4NO_3(21.51%) as compared to the control. During heading stage (Table 3b), the highest per cent increase in total chlorophyll content was found in treatment KNO_3 (39.03%) > $Ca(NO_3)_2$ (36.66%), and the lowest was in NH_4NO_3(23.42%) as compared to the control. In rice, nitrogen is an indispensable nutrient out of which 75% of leaf N is coupled with chloroplasts, and thus it is physiologically significant in production of dry matter by photosynthesis process (Dalling, 1985). Foliar application of nitrogen improved chlorophyll production in the leaves (Suwanarit and Sestapukdee, 1989). Peng *et al*. (2021) found that with rising nitrogen treatment rates, chlorophyll content is improved in leaves at the booting, heading, and maturity phase of rice. It suggested that nitrogen shortage might resulted in drop of chlorophyll content. Nitrogen foliar spray at later crop growth stages retarded the formation of abscisic acid, accelerated the cytokinin activity and resulted in higher chlorophyll retention. Thus, photosynthetic activity increased in leaves with the supply of photosynthates toward the grain and consequently resulted in higher yield of crops (Sarkar *et al*., 2007). Avila *et al.* (2021) revealed that potassium nitrate foliar application enhanced chlorophyll content, rate of photosynthesis, conductance of stomata, transpiration and carboxylation efficiency in sorghum plants. Oosterhuis and Bednarz (1997) concluded that potassium deficiency lowered the chlorophyll a and total chlorophyll concentration in cotton plants.

In the experiment, during maximum tillering stage (Table 4a), the highest per cent increase in nitrate reductase (NR) activity was found in treatment KNO_3 (95.92%) > $Ca(NO_3)_2$ (75.89%), and the lowest was in NH_4NO_3(64.21%) as compared to the control. During heading stage (Table 4b), the highest percent increase in total chlorophyll content was found in treatment KNO_3 (72.39%) > $Ca(NO_3)_2$ (59.42%), and the lowest was in NH_4NO_3(50.66%) as compared to the control(distilled water). Nitrate reductase is key regulator enzyme involved in nitrogen assimilation within the plants (Latifa and Anggarwulan, 2009). Nitrate reductase activity reflects the condition of nitrogen uptake and utilization within the plant (Liu *et al*., 2018). It was found that decline in NR activity may be due to low availability of substrate i.e. NO_3^- (Pal *et al.*, 2005). NR activity can be promoted by application of nitrogenous compound. A direct relation was observed between nitrate reductase activity and nitrogen content of leaf during water logging situation (Gomathi and Chandran, 2012). Chen and Huang *et al.* (2020) reported that in maize the NR activity was greater in nitrogen treated maize. Ahanger *et al.* (2015) stated that NR activity got enhanced by application of potassium nitrate in oats. Because, addition of potassium improves nitrogen utilization by depleting nitrogen contents from the medium and accompany of nitrate anion is necessary for the activity of NR activity.

TABLE 1

EFFECT OF NITROGEN AEROSOLS ON CHLOROPHYLL 'A'CONTENT IN LEAF AT DIFFERENT GROWTH STAGES OF RICE CROP

Treatments / Varieties	(a) Chlorophyll 'a' content (mg g^{-1}fw of leaf) at maximum tillering stage					(b) Chlorophyll 'a' content (mg g^{-1}fw of leaf) at heading stage				
	N-aerosols (60 Kg ha^{-1})					N-aerosols (60 Kg ha^{-1})				
	Control	KNO_3	NH_4NO_3	$Ca(NO_3)_2$	Mean	Control	KNO_3	NH_4NO_3	$Ca(NO_3)_2$	Mean
Aghonibora	0.61	0.64	0.62	0.68	0.64	0.72	0.91	0.74	0.77	0.78
Gitesh	0.58	0.66	0.63	0.68	0.64	0.82	0.89	0.84	0.86	0.85
Aki Sali	0.48	0.6	0.51	0.58	0.54	0.67	0.77	0.72	0.75	0.72
Prafulla	0.56	0.64	0.62	0.65	0.62	0.73	0.82	0.76	0.79	0.78
Solpana	0.48	0.55	0.51	0.56	0.52	0.62	0.76	0.66	0.71	0.68
Bahadur	0.56	0.61	0.59	0.62	0.59	0.72	0.81	0.74	0.77	0.76
Ranjit	0.62	0.68	0.66	0.72	0.66	0.76	0.86	0.81	0.83	0.82
Swarna sub1	0.46	0.52	0.5	0.54	0.5	0.48	0.59	0.52	0.58	0.54
Chahou	0.28	0.36	0.31	0.38	0.33	0.36	0.53	0.39	0.51	0.44
Shruboni	0.22	0.44	0.28	0.46	0.34	0.38	0.55	0.42	0.54	0.47
Mean	0.48	0.56	0.52	0.58		0.62	0.74	0.66	0.71	
	Factor	S.Ed(±)	CD (0.05)			Factor	S.Ed(±)	CD (0.05)		
	Varieties (A)	0.02	0.04			Varieties (A)	0.02	0.03		
	Aerosols (B)	0.01	0.02			Aerosols (B)	0.01	0.02		
	A×B	0.04	NS			A×B	0.03	NS		

TABLE 2

EFFECT OF NITROGEN AEROSOLS ON CHLOROPHYLL 'B' CONTENT IN LEAF AT DIFFERENT STAGES OF RICE CROP

Treatments / Varieties	(a) Chlorophyll 'b' content (mg g^{-1}fw of leaf) at maximum tillering stage					(b) Chlorophyll 'b' content (mg g^{-1}fw of leaf) at heading stage				
	N-aerosols (60 Kg ha^{-1})					N-aerosols (60 Kg ha^{-1})				
	Control	KNO_3	NH_4NO_3	$Ca(NO_3)_2$	Mean	Control	KNO_3	NH_4NO_3	$Ca(NO_3)_2$	Mean
Aghonibora	1.66	1.76	1.71	1.72	1.71	1.74	1.94	1.84	1.82	1.8
Gitesh	1.69	1.78	1.72	1.76	1.74	1.76	1.92	1.86	1.88	1.84
Aki Sali	1.57	1.66	1.61	1.64	1.62	1.68	1.8	1.71	1.775	1.74
Prafulla	1.61	1.72	1.64	1.67	1.66	1.75	1.84	1.78	1.82	1.79
Solpana	1.54	1.63	1.56	1.61	1.58	1.64	1.78	1.68	1.73	1.71
Bahadur	1.61	1.69	1.63	1.66	1.64	1.74	1.84	1.76	1.79	1.78
Ranjit	1.64	1.74	1.67	1.71	1.69	1.78	1.86	1.82	1.84	1.82
Swarna sub1	1.46	1.6	1.52	1.54	1.53	1.48	1.61	1.52	1.61	1.54
Chahou	1.28	1.37	1.31	1.39	1.34	1.46	1.55	1.48	1.52	1.5
Shruboni	1.22	1.44	1.28	1.46	1.34	1.48	1.57	1.51	1.56	1.52
Mean	1.52	1.64	1.56	1.61		1.66	1.77	1.68	1.74	
	Factor	S.Ed(±)	CD (0.05)			Factor	S.Ed(±)	CD (0.05)		
	Varieties (A)	0.02	0.04			Varieties (A)	0.009	0.02		
	Aerosols (B)	0.01	0.02			Aerosols (B)	0.006	0.01		
	A×B	0.03	NS			A×B	0.017	0.04		

TABLE 3

EFFECT OF NITROGEN AEROSOLS ON TOTAL CHLOROPHYLL CONTENT IN LEAF AT DIFFERENT STAGES OF RICE CROP

Treatments / Varieties	(a) Total chlorophyll content (mg g^{-1}fw of leaf) at maximum tillering stage					(b) Total chlorophyll content (mg g^{-1}fw of leaf) at heading stage				
	N-aerosols (60 Kg ha^{-1})					N-aerosols (60 Kg ha^{-1})				
	Control	KNO_3	NH_4NO_3	$Ca(NO_3)_2$	Mean	Control	KNO_3	NH_4NO_3	$Ca(NO_3)_2$	Mean
Aghonibora	1.83	2.62	2.01	2.55	2.25	2.02	2.74	2.31	2.39	2.41
Gitesh	1.79	2.48	1.82	2.45	2.13	2.41	3.05	2.52	2.43	2.72
Aki Sali	1.52	1.9	1.6	1.88	1.72	1.73	1.98	1.79	2.11	1.93
Prafulla	1.72	2.59	2.09	2.36	2.19	1.87	2.6	2.02	2.25	2.23
Solpana	1.49	1.78	1.56	1.74	1.64	1.72	1.95	1.78	2.07	1.93
Bahadur	1.53	2.4	1.7	2.2	1.95	1.75	2.25	2.16	2.2	2.14
Ranjit	1.89	2.91	2.09	2.54	2.35	1.99	2.67	2.17	2.35	2.33
Swarna sub1	1.48	1.65	1.49	1.5	1.53	1.66	1.88	1.74	2.1	1.87
Chahou	1.36	1.51	1.4	1.44	1.43	1.5	1.75	1.6	2.05	1.72
Shruboni	1.43	1.56	1.46	1.45	1.47	1.66	1.88	1.74	2.08	1.87
Mean	1.61	2.14	1.71	2.01		1.83	2.27	1.98	2.2	
	Factor	S.Ed(±)	CD (0.05)			Factor	S.Ed(±)	CD (0.05)		
	Varieties (A)	0.02	0.05			Varieties (A)	0.04	0.09		
	Aerosols (B)	0.02	0.03			Aerosols (B)	0.03	0.06		
	A×B	0.05	0.11			A×B	0.09	0.19		

TABLE 4

EFFECT OF NITROGEN AEROSOLS ON NITRATE REDUCTASE (NR) ACTIVITY IN LEAF AT DIFFERENT STAGES OF RICE CROP

Treatments / Varieties	(a) NR activity (nmol NO_2^- g^{-1} fw of leaf h^{-1}) at maximum tillering stage					(b) NR activity (nmol NO_2^- g^{-1} fw of leaf h^{-1}) at heading stage				
	N-aerosols (60 Kg ha^{-1})					N-aerosols (60 Kg ha^{-1})				
	Control	KNO_3	NH_4NO_3	$Ca(NO_3)_2$	Mean	Control	KNO_3	NH_4NO_3	$Ca(NO_3)_2$	Mean
Aghonibora	15.95	22.31	18.84	18.18	18.82	19.26	28.31	25.2	28.07	25.21
Gitesh	13.15	22.56	19.52	18.81	18.51	12.07	22.33	19.82	21.23	18.86
Aki Sali	12.1	20.86	18.23	19.29	17.62	12.02	20.62	17.69	19.05	17.34
Prafulla	14.12	22.62	19.64	19.53	18.97	13.19	23.08	20.92	20.74	19.48
Solpana	11.19	19.08	15.59	17.55	15.85	10.95	19.12	15.18	17.08	15.58
Bahadur	16.94	26.02	20.58	23	21.63	17.42	27.68	24.7	28.19	24.5
Ranjit	14.27	21.08	17.72	18.79	17.96	15.12	25.69	22.01	23.32	21.53
Swarna sub1	12.17	18.31	13.16	15.65	14.82	11.71	18.23	12.86	15.58	14.59
Chahou	11.47	17.13	12.29	13.42	12.58	8.83	17.3	11.51	14.08	12.93
Shruboni	11.81	17.09	12.98	15.35	13.81	10.68	16.82	12.84	14.77	13.77
Mean	12.72	20.71	16.85	17.95		13.12	21.92	18.28	20.21	
	Factor	S.Ed(±)	CD (0.05)			Factor	S.Ed(±)	CD (0.05)		
	Varieties (A)	0.45	0.92			Varieties (A)	0.32	0.66		
	Aerosols (B)	0.28	0.58			Aerosols (B)	0.21	0.42		
	A×B	0.91	1.84			A×B	0.65	1.32		

TABLE 5

EFFECT OF NITROGEN AEROSOLS ON LIPID PEROXIDATION IN LEAF AT DIFFERENT GROWTH STAGES OF RICE CROP

Treatments / Varieties	(a) Lipid peroxidation (nmol MDA g^{-1}fw) at maximum tillering stage					(b) Lipid peroxidation (nmol MDA g^{-1} fw)at heading stage				
	N-aerosols (60 Kg ha^{-1})					N-aerosols (60 Kg ha^{-1})				
	Control	KNO_3	NH_4NO_3	$Ca(NO_3)_2$	Mean	Control	KNO_3	NH_4NO_3	$Ca(NO_3)_2$	Mean
Agnibora	0.57	0.68	1.18	0.61	0.76	1.02	1.06	1.11	1.04	1.06
Gitesh	0.56	0.57	1.16	0.59	0.72	1.02	1.06	1.08	1.04	1.05
Aki Sali	0.62	0.74	1.23	0.68	0.81	1.09	1.12	1.13	1.11	1.11
Prafulla	0.6	0.7	1.21	0.66	0.79	1.04	1.11	1.13	1.06	1.08
Solpana	0.63	0.68	1.24	0.66	0.8	1.13	1.2	1.23	1.16	1.18
Bahadur	0.61	0.62	1.18	0.66	0.76	1.05	1.12	1.17	1.07	1.1
Ranjit	0.59	0.68	1.19	0.64	0.77	1.04	1.08	1.13	1.06	1.08
Swarna sub1	0.66	0.81	1.31	0.75	0.88	1.11	1.15	1.21	1.12	1.14
Chahou	0.64	0.72	1.21	0.61	0.79	1.06	1.15	1.18	1.11	1.12
Shruboni	0.66	0.79	1.23	0.68	0.84	1.12	1.14	1.22	1.14	1.15
Mean	0.61	0.69	1.21	0.65		1.07	1.12	1.16	1.09	
	Factor	S.Ed(±)		CD (0.05)		Factor	S.Ed(±)		CD (0.05)	
	Varieties (A)	0.02		0.04		Varieties (A)	0.005		0.011	
	erosols (B)	0.02		0.03		Aerosols (B)	0.003		0.007	
	A×B	0.04		NS		A×B	0.011		0.022	

TABLE 6

EFFECT OF NITROGEN AEROSOLS ON CELL MEMBRANE STABILITY (CMS) IN LEAF AT MAXIMUM AT DIFFERENT STAGES OF RICE CROP

Treatments / Varieties	(a) CMS at maximum tillering stage					(b) CMS at heading stage				
	N-aerosols (60 Kg ha^{-1})					N-aerosols (60 Kg ha^{-1})				
	Control	KNO_3	NH_4NO_3	$Ca(NO_3)_2$	Mean	Control	KNO_3	NH_4NO_3	$Ca(NO_3)_2$	Mean
Aghonibora	4.45	4.99	4.71	5.12	4.82	5.16	5.98	5.57	6.3	5.75
Gitesh	4.64	5.01	4.86	5.15	4.91	7.23	7.58	7.37	7.39	7.39
Aki Sali	4.03	4.15	4.07	4.25	4.12	6.23	7.78	6.62	7.72	7.09
Prafulla	4.61	4.89	4.71	5.03	4.81	7.39	8.42	7.67	9.18	8.16
Solpana	3.96	4.25	4.11	4.32	4.16	6.31	7.2	6.58	7.25	6.83
Bahadur	6.23	6.64	6.43	7.25	6.63	6.27	6.76	6.24	7.17	6.61
Ranjit	4.66	4.91	4.81	5.02	4.85	6.54	7.55	7.24	7.68	7.25
Swarna sub1	4.12	4.16	4.14	4.55	4.24	5.51	6.65	5.67	5.79	5.91
Chahou	3.71	3.91	3.89	4.16	3.92	5.36	6.22	5.55	6.13	5.82
Shruboni	3.71	3.92	3.75	4.09	3.86	4.84	5.7	5.23	6.28	5.51
Mean	4.41	4.68	4.54	4.89		5.88	6.78	6.17	6.99	
	Factor	S.Ed(±)		CD (0.05)		Factor	S.Ed(±)		CD (0.05)	
	Varieties (A)	0.22		0.45		Varieties (A)	0.005		0.011	
	Aerosols (B)	0.14		0.28		Aerosols (B)	0.003		0.007	
	A×B	0.45		NS		A×B	0.011		0.022	

TABLE 7

EFFECT OF NITROGEN AEROSOLS ON NITROGEN AND PROTEIN CONTENTS IN GRAIN AT HARVEST STAGE OF RICE CROP

Treatments / Varieties	(a) Total nitrogen contents (%) in grain					(b) Crude Protein content (%) in grains				
	N-aerosols (60 Kg ha^{-1})					N-aerosols (60 Kg ha^{-1})				
	Control	KNO_3	NH_4NO_3	$Ca(NO_3)_2$	Mean	Control	KNO_3	NH_4NO_3	$Ca(NO_3)_2$	Mean
Aghonibora	1.43	1.51	1.45	1.55	1.48	9.28	9.66	9.52	9.81	9.55
Gitesh	1.56	1.62	1.59	1.58	1.59	10.03	10.59	10.19	10.88	10.42
Aki Sali	1.34	1.42	1.36	1.52	1.41	8.36	8.81	8.59	9.36	8.78
Prafulla	1.4	1.45	1.49	1.56	1.48	8.98	9.25	9.2	9.55	9.24
Solpana	1.25	1.34	1.28	1.44	1.32	7.94	8.61	8.43	9.42	8.6
Bahadur	1.38	1.44	1.41	1.54	1.44	8.69	9.06	8.85	9.58	9.04
Ranjit	1.38	1.45	1.46	1.55	1.46	8.94	9.25	9.25	9.7	9.28
Swarna sub1	1.28	1.38	1.34	1.46	1.36	8.01	8.58	8.32	9.11	8.5
Chahou	1.15	1.34	1.3	1.41	1.3	7.19	8.36	8.16	8.68	8.1
Shruboni	1.24	1.36	1.29	1.39	1.32	7.6	8.44	8.22	8.8	8.27
Mean	1.34	1.43	1.39	1.5		8.5	9.06	8.87	9.48	
	Factor	S.Ed(±)	CD (0.05)			Factor	S.Ed(±)	CD (0.05)		
	Varieties (A)	0.02	0.04			Varieties (A)	0.15	0.32		
	Aerosols (B)	0.01	0.02			Aerosols (B)	0.10	0.21		
	A×B	0.04	NS			A×B	0.32	NS		

In the experiment, during maximum tillering stage (Table 5a), the highest per cent increase in lipid peroxidation was brought about by NH_4NO_3(107.14%) followed by KNO_3 (22.72%), and the lowest was in case of $Ca(NO_3)_2$ (13.63%) as compared to the control. During heading stage (Table 5b), the highest per cent increase in lipid peroxidation was found in treatment NH_4NO_3(11.42%) followed by KNO_3 (8.49%), and the lowest was in $Ca(NO_3)_2$ (4.71%) as compared to the control(distilled water). Plant's stress condition can be monitored by a marker process known as lipid peroxidation (Bojtor *et al.*, 2019). Lipid peroxidation causes cell death and oxidative damage to cell structures by creating toxicity inside the cell (Bharali *et al*., 2015a). Because, lipid peroxidation occurs due to formation of reactive nitrogen species (ROS). Kong *et al.* (2017) reported that excessive nitrogen application caused oxidative stress in plants because of inactivation of antioxidatant enzymes and reduction in metabolites which plays role in scavenging ROS. Additionally, nitrogen and lipid metabolism also got disrupted. As a consequence, rate of grain filling is interrupted, leaf senescence is accelerated and eventually it reduces the grain yield. Campos *et al.* (2016) stated that ammonium ion in ammonium nitrate enhanced the production of reactive oxygen species in cucumber plant which resulted in decreased growth, chlorosis and necrosis of cucumber roots and leaves. Jan *et al.* (2019) proposed that potassium nitrate-maintained turgidity and lowered the deleterious effects of reactive oxygen species. Plant responses to environmental stresses were found to be regulated by calcium which has important role in cell wall and cell membrane stabilization (Ranty *et al*., 2006). In *Oryza sativa* L. and *Hordum vulgare* L., it has been reported that potassium nitrate augmentation caused reduction of MDA content (Yu-Chuan *et al.*, 2008; Hafsi *et al.*, 2010).

In the experiment, during maximum tillering stage (Table 6a), the highest per cent increase in CMS was found in treatment $Ca(NO_3)_2$ (14.92%) > KNO_3 (12.01%), and the lowest was in NH_4NO_3 (5.72%) as compared to the control. During heading stage (Table 6b), the highest per cent increase in lipid peroxidation was found in treatment $Ca(NO_3)_2$ (29.85%) > KNO_3 (24.95%), and the lowest was in NH_4NO_3(10.69%) as compared to the control(distilled water). Cell membrane stability determines the amount of leakage of electrolytes from the segment of leaf (Beltrano and Ronco, 2008). The membrane stability maintenance during the period of stress was found to be important for usual physiological metabolism to persist during reduced water potential (Tripathy *et al.*, 2000). In a study, the cation leakage was found to be greater by the influence of ammonium ions due to which cation's quantity was larger in the both intercellular and exchangeable locations and thus caused in reduction in the stability of the membrane in rice (Bharali *et al.*, 2015b). There is also possibility that lipid peroxidation got accelerated in ammonium treated tissues which is mainly due to more ROS production by ammonium ions (Zhu *et al.*, 2000; Hachiya *et al.*, 2010; Patterson *et al.*, 2010).

In the experiment, the highest per cent increase in intercellular [K^+] concentration in cell at maximum tillering (Fig 1a) stage was found in treatment KNO_3 (59.04%) followed by $Ca(NO_3)_2$ (44.76%), and the lowest was in NH_4NO_3(31.42%) as compared to the control while in the highest per cent increase in exchangeable [K^+] concentration in cell at maximum tillering stage (Fig 1b) was found in treatment KNO_3 (68.86%) followed by $Ca(NO_3)_2$ (74.76%), and the lowest was in NH_4NO_3 (71.02%) as compared to the control(distilled water).

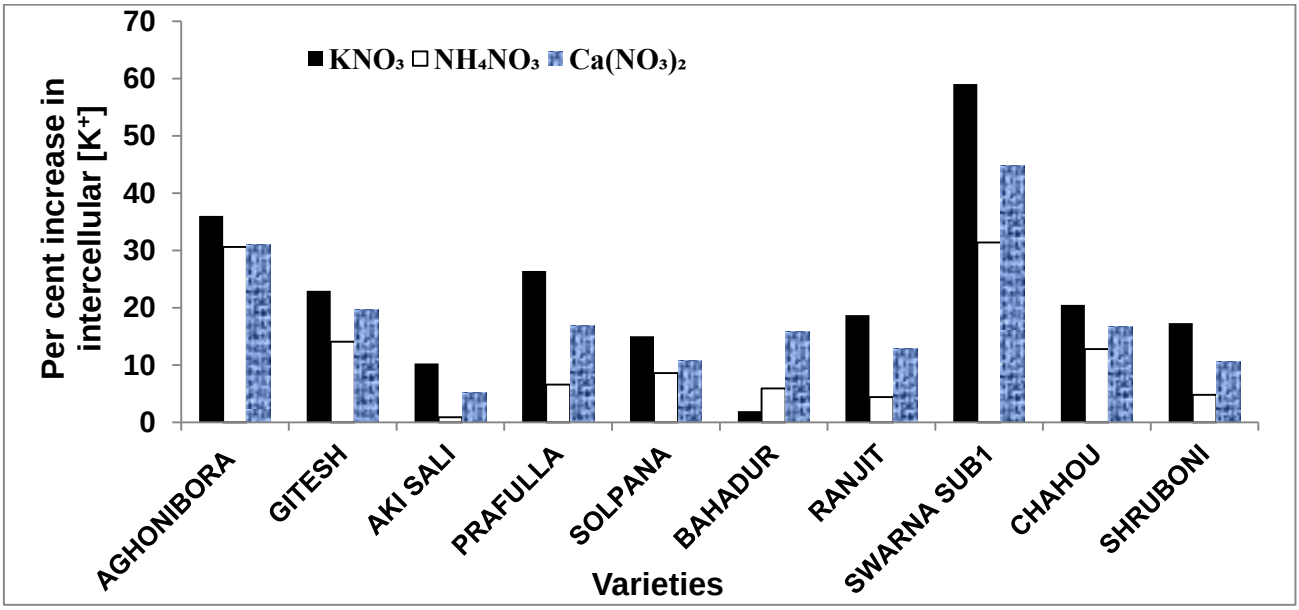

FIGURE 1 (a): Change in intercellular [K+] of rice leaf at maximum tillering stage as affected by nitrogen aerosols as compared to control

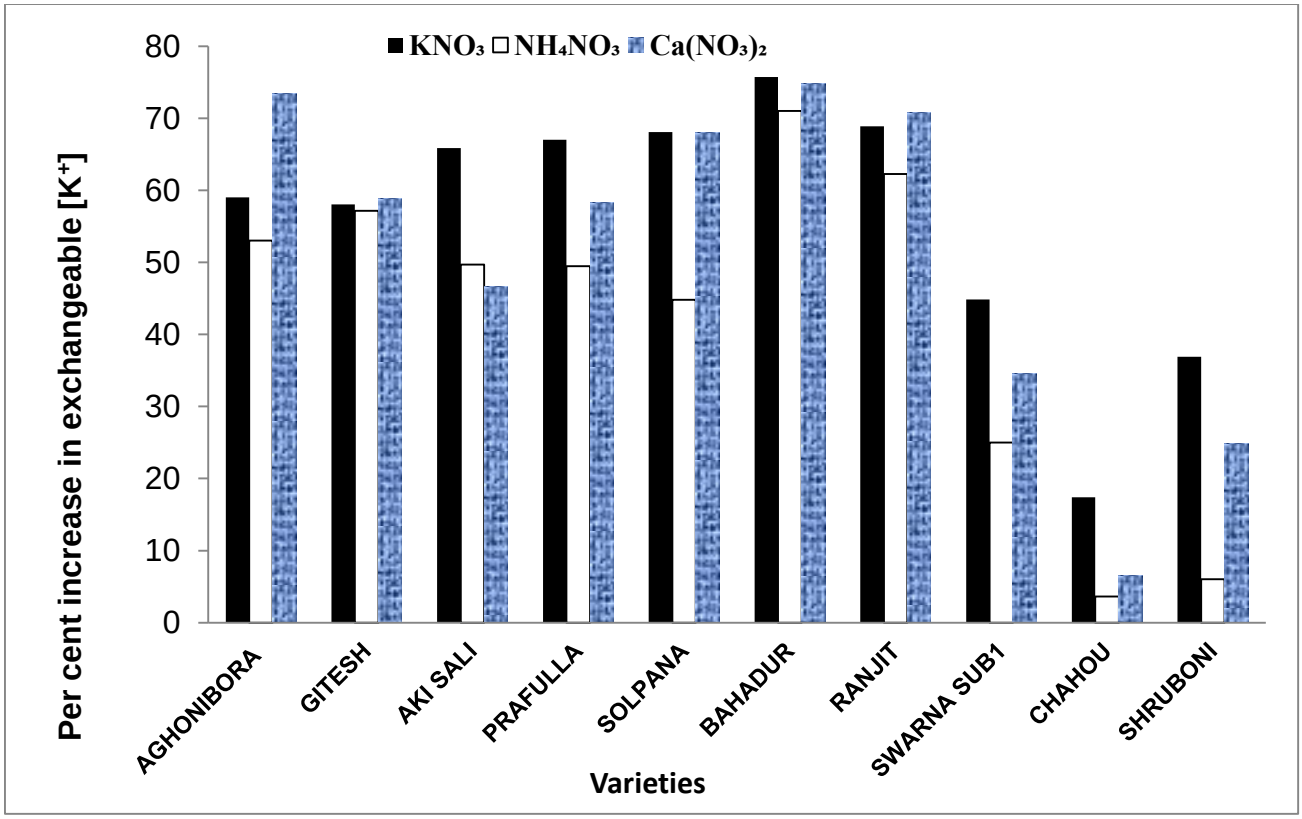

FIGURE 1 (b): Changes in exchangeable [K⁺] in rice at maximum tillering stage as affected by aerosols treatments as compared to control

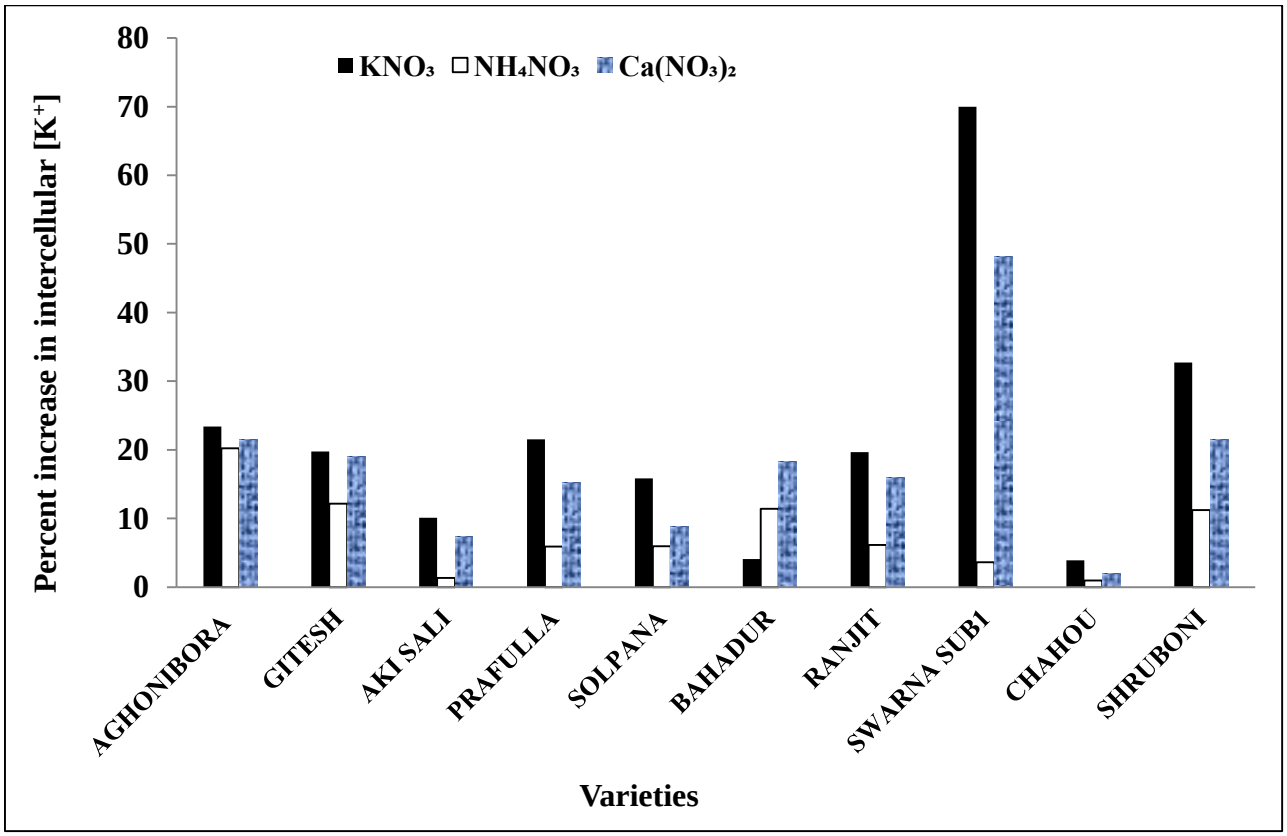

FIGURE 2 (a): Change in intercellular [K⁺] in leaf of rice at heading stage as affected by nitrogen aerosols as compared to control

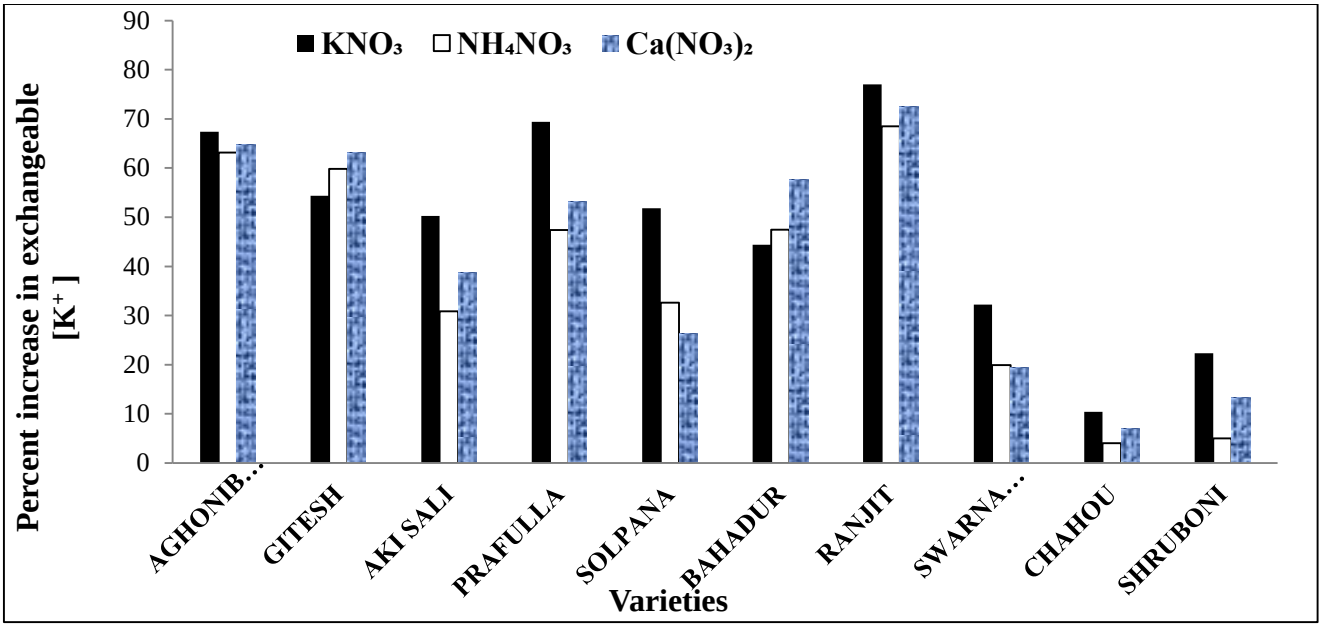

FIGURE 2 (b): Change in exchangeable [K^+] in leaf of rice at heading stage as affected by aerosols in comparison to control

In the experiment (Fig.2a), the highest percent increase in intercellular [K^+] concentration in cell at heading stage was found in treatment KNO_3 (23.41%) followed by $Ca(NO_3)_2$ (48.18%), and the lowest was in NH_4NO_3 (20.23%) as compared to the control while the highest per cent increase in exchangeable [K^+] concentration in cell at heading stage (Fig 2b) were found in treatment KNO_3 (77.02%) followed by $Ca(NO_3)_2$ (72.52%), and the lowest was in NH_4NO_3(68.46%) as compared to the control(distilled water). Ammonium and nitrite aerosols caused damaged to membrane which made membrane leaky for the cations and thus concentrations of cations became higher in intercellular and extracellular locations irrespective of plant varieties (Bharali *et al.*, 2015b). Potassium nitrate plays a key role in activation of various enzymes, supporting of electrical potential gradients across cell membranes. It is also indispensable for photosynthesis process, protein synthesis, and regulation of stomatal movement, and is the major cation in the maintenance of cation-anion balance (Marschner, 1995). Potassium has impact on enzymes required for conversion of atmospheric nitrogen to ammonia in rhizobium. Potassium nitrate was found to enhance carbohydrate supply for reduction of nitrogen inside the nodules for amino acid synthesis (Havilan *et al.*, 2005).

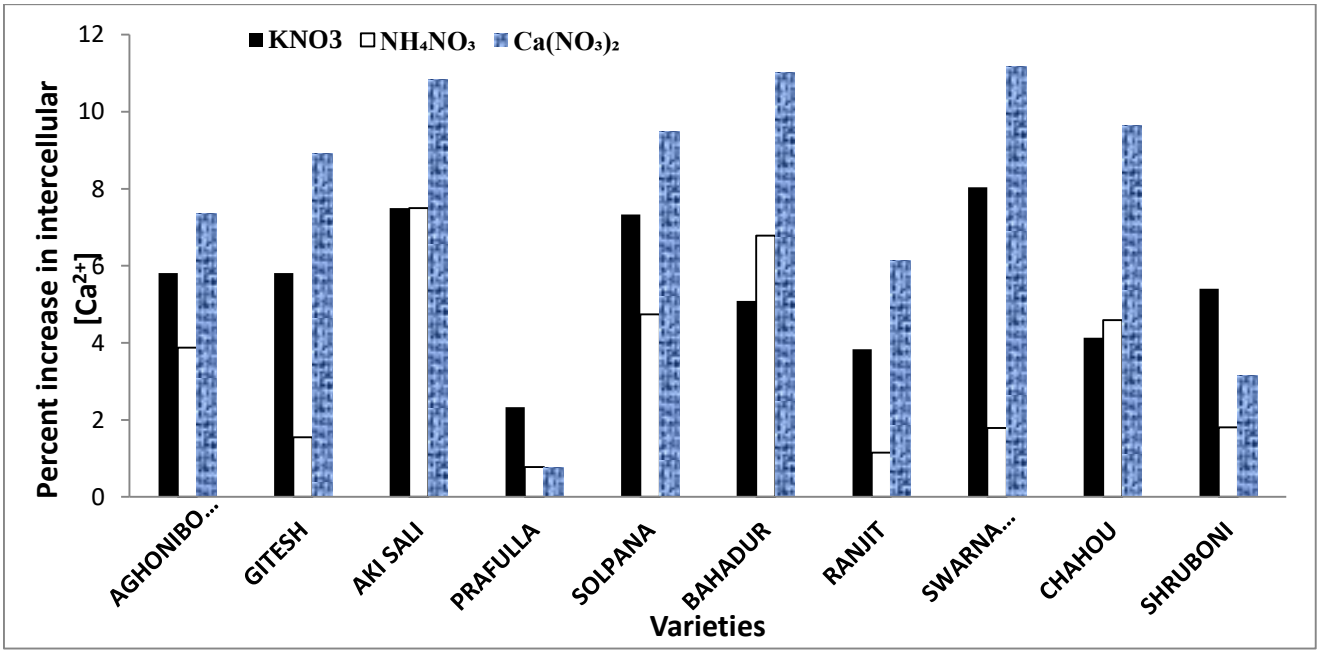

FIGURE 3 (a): Changes in intercellular [Ca^{2+}] in leaf of rice at maximum tillering stage as affected by N-aerosols in comparison to control

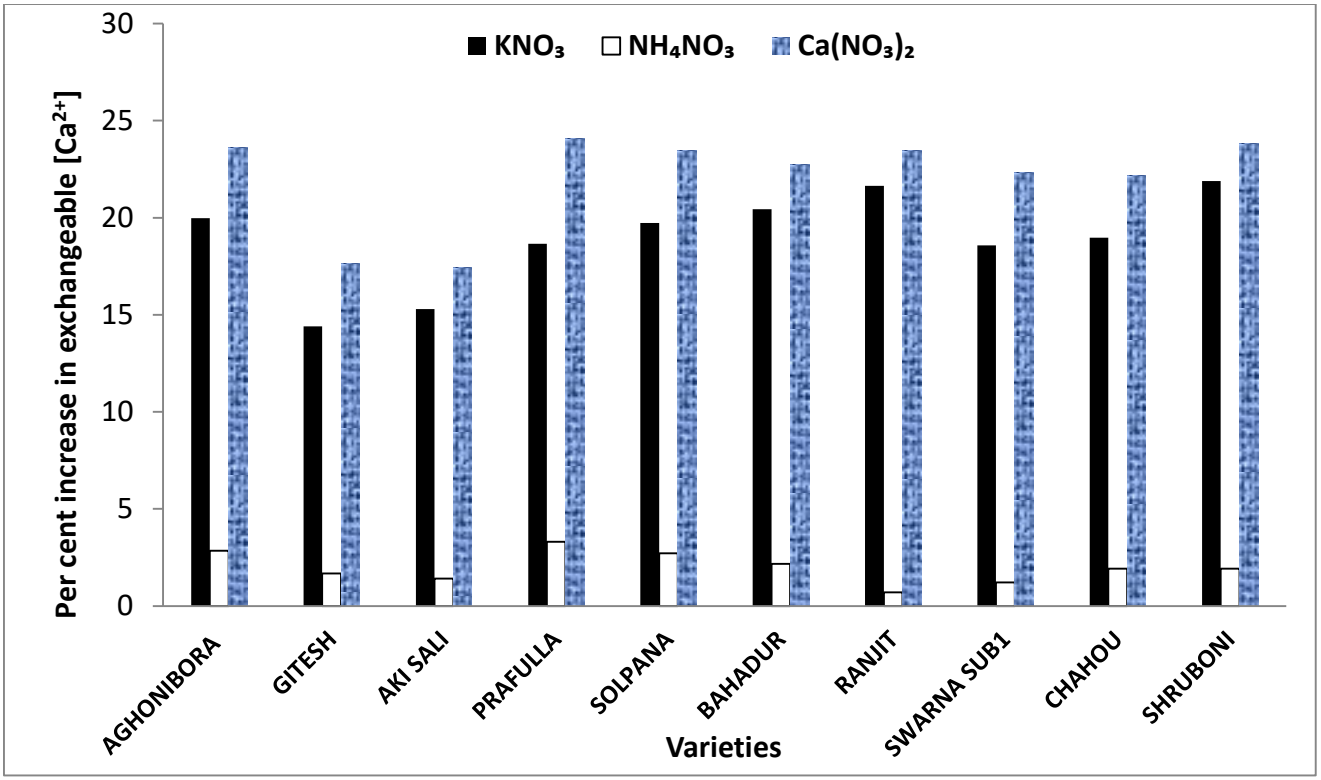

FIGURE 3 (b): Change in exchangeable [Ca^{2+}] in leaf of rice at maximum tillering stage as affeted by aerosol in comparison to control

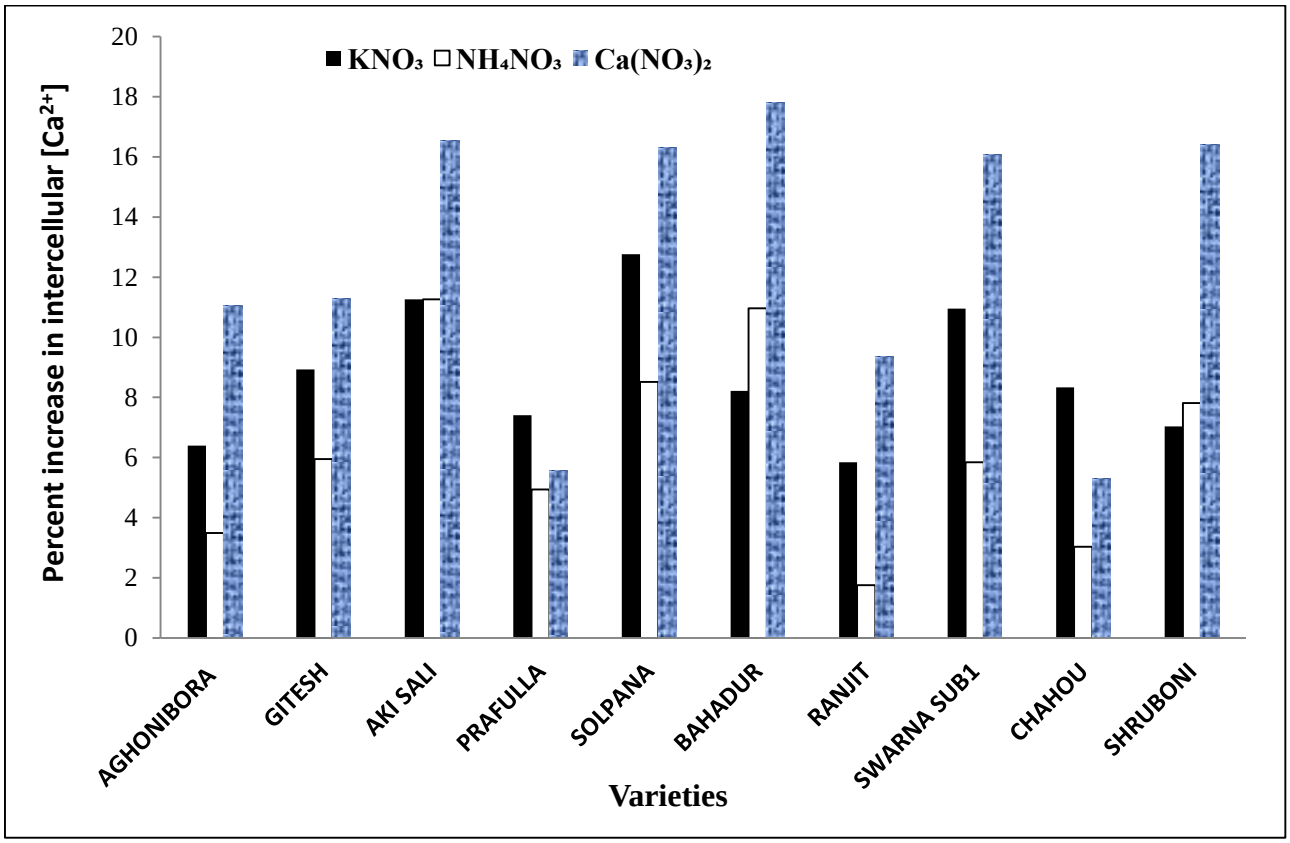

FIGURE 4 (a): Changes in intercellular [Ca^{2+}] in leaf of rice at heading stage as affected by N-aerosol in comparison to control

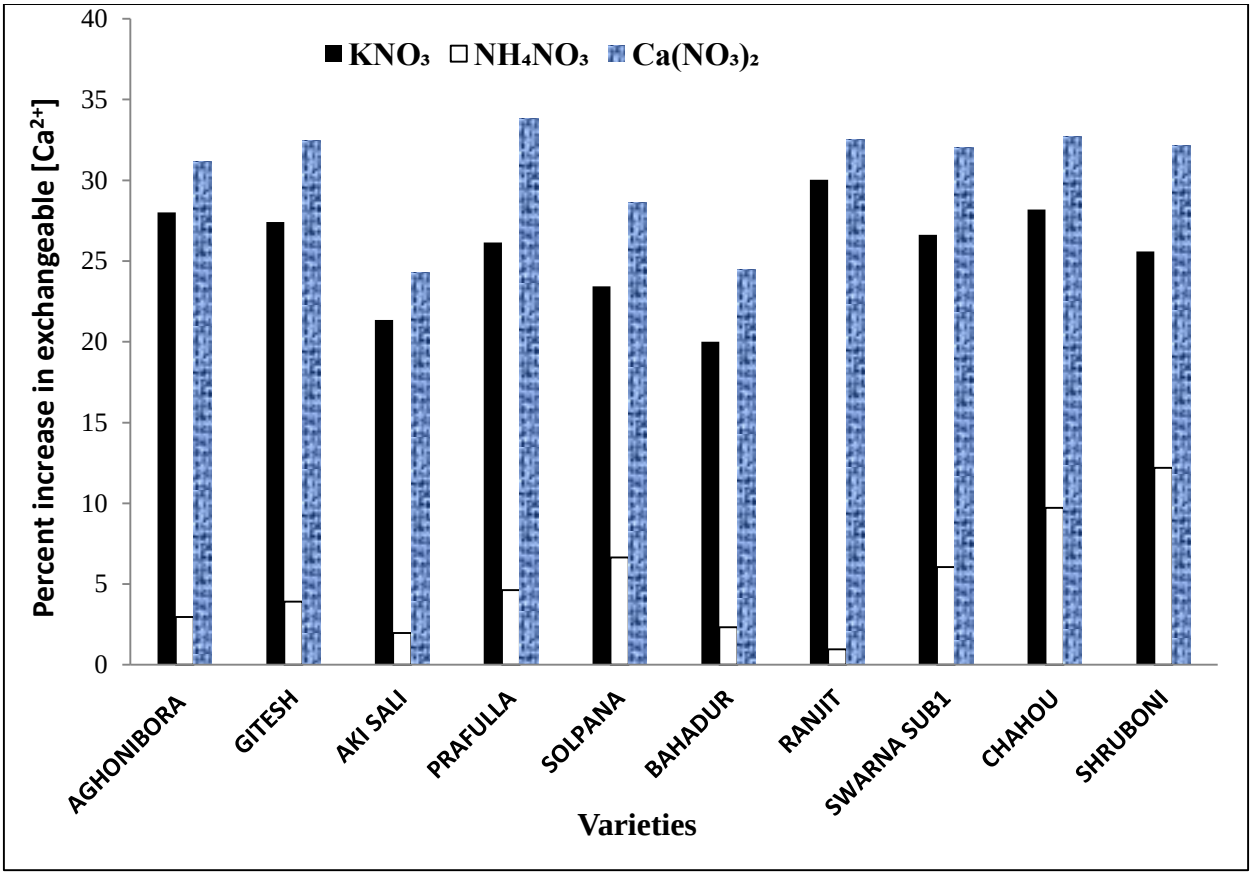

FIGURE 4(b): Changes in exchangeable [Ca^{2+}] in leaf of rice at heading stage as affected by N-aerosol in comparison to control

In the experiment, during maximum tillering stage (Fig 3a) the highest per cent increase in intercellular [Ca^{2+}] concentration in cell was found in treatment $Ca(NO_3)_2$ (11.16%) > KNO_3 (8.03%), and the lowest was in NH_4NO_3 (6.77%) as compared to the control while the highest per cent increase in exchangeable [Ca^{2+}] concentration (Fig 3b) in cell was found in treatment Ca $(NO_3)_2$ (23.80%) > KNO_3 (21.88%), and the lowest was in NH_4NO_3 (3.29%) as compared to the control(distilled water).

In the experiment, heading stage the highest per cent increase in intercellular [Ca^{2+}] concentration (Fig 4a) in cell was found in treatment $Ca(NO_3)_2$ (17.80%) > KNO_3 (12.76%), and the lowest was in NH_4NO_3(11.25%) as compared to the control while, the highest per cent increase in exchangeable [Ca^{2+}] concentration (Fig 4b) in cell was found in treatment Ca $(NO_3)_2$ (33.80%) > KNO_3 (30.03%), and the lowest was in NH_4NO_3(12.21%) as compared to the control(distilled water). Calcium is essential for maintaining the strength of cell walls, growth and division of cell, assimilation of nitrogen and working as enzyme cofactors (Sajid *et al.,* 2020). Calcium nitrate aerosol supply calcium ions to plants which play important role in protecting the membrane from damage caused due to peroxidation of membranes and solute leakage and enhance the CMS within the plant cells (Borgohain *et al.,* 2019). Marschner (1995) reported that nitrogen foliar nutrition resulted in reduction in membrane permeability but adding up of Mg^{2+} and Ca^{2+} ions along with nitrogen application to plants and improved the integrity of membrane because of calcium which maintains the structural and functional integrity of plant membrane, stabilize cell wall structure, regulate ion transport and selectivity and control ion exchange behavior as well as enzyme activities.

In the experiment, during harvesting stage (Table 7a), the highest per cent increase in total nitrogen content in grains was found in treatment $Ca(NO_3)_2$ (22.61%) > KNO_3 (16.52%), and the lowest was in NH_4NO_3(13.04%) as compared to the control (distilled water). The capacity of plant to translocate nitrogen into grains is considered as sink while the supply of N that is available for translocation into the grain is considered as source, and also these factors are thought to be governing the nitrogen content of grains (Kato, 2012). Lyu *et al.* (2022) stated that nitrogen foliar application fosters N remobilization and N uptake following anthesis by increasing the activity of grain N metabolism enzymes like NR, Glutamine synthetase (GS) and glutamate pyruvate transaminase (GPT) and protein accumulation particularly during the period of middle and late grain-filling stages. These in turn caused the grain N demand and remobilization of nitrogen, directing to greater grain protein content and grain quality. Application of nitrogen during the fruiting stage could boostup wheat's protein content from 10.8 to 21.0% (Woolfolk

et al., 2002). Kaya *et al*. (2003) reported that $Ca(NO_3)_2$ foliar spray improved the content of calcium and nitrogen in cucumber and melon plants. Calcium nitrate is highly soluble in nature and its high solubility makes it popular for immediate supply of available source of nitrate and calcium directly to soil through irrigation water or with foliar applications. Tripathy *et al.* (2018) suggested that Ca^{2+} presence along with nitrate improves the utilization of nitrogen and greater assimilation of nitrate in roots and leaves.

In the experiment, during harvesting stage (Table 7b), the highest percent increase in crude protein content in grains was found in treatment $Ca(NO_3)_2$ (20.72%) > KNO_3 (16.27%), and the lowest was in NH_4NO_3(13.49%) as compared to the control(distilled water). Tripathy *et al.* (2018) showed the significant effect of treatment ofNO_3^- with the pair of counter ions (K^+ and Ca^{2+}) by increasing grain protein content and chlorophyll synthesis in hybrid rice. Yathish *et al*. (2021) stated that foliar treatment of calcium nitrate enhanced nitrogen and protein content in grains due to greater uptake of calcium cation which played a crucial role in utilization of nitrogen present in soil and assimilation of nitrate in leaves and roots of pigeonpea. Vijayakumar *et al.* (2022) reported that potassium nitrate foliar application promotes the amino acid translocation from vegetative parts to grains as well as conversion of amino acids to grain proteins.

IV. CONCLUSIONS

- Among the nitrogen aerosols, KNO_3 was observed to be more effective than $CaNO_3$ and NH_4NO_3 as compared to the control (distilled water). Because, KNO_3 was found to support K and N-nutrition as well as maintained the cell membrane stability with higher retention of intercellular and exchangeable ions and greater enzymatic (NR) activity in rice.
- All the N-aerosols @60 $Kgha^{-1}$ impacted majority of the biochemical traits of rice crop positively.
- Among the 10 rice varieties, Gitesh was found to be the best as it possessed the desirable characteristics under the influence of foliar nitrogen aerosols application.
- In the present investigation, variety Gitesh can be considered prominent one in terms of foliar K-nutrition. This variety may be explored in rice improvement programme in the current climate change scenarios globally.

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