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INDUCED-ANTIFUNGAL ACTIVITY IN *ORYZA SATIVA* L. CAUSED BY *PYRICULARIA ORYZAE* CAV. WITH A LOW DOSE OF NICKEL PRIMING

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Abstract

Hydroponically grown rice seedlings were treated with a micronutrient Ni (II) and blast inducing fungus *Pyricularia oryzae* Cav. (MTCC 1477) under controlled condition to investigate the effect of Ni (II) on *Pyricularia oryzae* Cav. (MTCC 1477) in *Oryza sativa* L. (var. Naveen). Ten days old seedlings were treated with different Ni (II) (as $NiCl_2$) concentrations (40 µM) and after 3 days, spores of *P. oryzae* were sprayed on these treated seedlings each of 2 mL (contains 10^5 CFU/mL). The morphological, biochemical and chlorophyll fluorescence parameters were studied in 10, 20 and 30d treated seedlings to evaluate the effect of Ni (II) on fungus in different gradations and effect of co-stress on *Oryza sativa* L. There was a significant decrease in growth and physio-chemical parameters in both the fungus (F) and Ni (II) treated rice seedlings when grown separately, whereas in co-stress of fungus with Ni (II) (40 µM) treated seedlings these parameters were restored. It indicates that Ni (II) inhibits the activity of fungal toxins in the rice seedlings. The JIP-test parameters and the anti-oxidative enzyme activity showed a growth-enhancing effect for 40 µM Ni (II) diseased rice plants. Under present experimental conditions, the study suggests that 40 µM Ni (II) concentration act as a nutritive supplement as well as induces antifungal activity against *P. oryzae* Cav. causing rice blast disease in *O. Sativa* L.

Key words : Antioxidative enzymes, Chlorophyll fluorescence parameters, Nickel (II), *Oryza sativa*, *Pyricularia oryzae*.

Introduction

Plants have developed different mechanisms for their survival sensing the external stress environment, get stimulated, and then create cellular responses accordingly to cope and combat abiotic or biotic stress. The signalling pathways have a vital role in sensing the environment stress and accordingly produce a distinct physiological and biochemical response (Zhu 2002). Plants under heavy metal stress are more prone to diseases by herbivores and/or microbial infections. Sometimes negative, positive or neutral effect between metal and pathogenic infection may experience by many plants (Hanson *et al.,* 2003; Jhee *et al.,* 2005). Heavy metal stress might save energy-demanding organic fortifications (Boyd and Martens 1998). But some heavy metals may induce resistance against biotic stress in plants. Metal ions may initiate biochemical reactions and rarely fight against pathogenic disease in non-hyper-accumulator plants (Walters *et al.,* 2005). But, metal-induced ROS that can set off protection signals and as a result fabricates secondary metabolites (Jiang *et al.,* 2005).

Blast is one of the dominant airborne or seed-borne infections in rice across the globe. The pathogen *Pyricularia oryzae* Cav. usually causes leaf blast or neck blast (Bonman 1992). It was a reported fact that the productivity of rice significantly drops due to blast disease in 75% of the cases. Higher tolerance to excess metal of the plant than of the pathogen can lead to hermetic response where growth stimulation can be observed due to the efficient repression of the pathogen (Calabrese *et al.,* 2007).

Nickel being a micronutrient required by many plant in traces whereas in excess it induces heavy metal stress response. The present study aims to induce an optimal dose of Ni-stress which may trigger many genes encoding certain metabolites responsible to detoxify the fungal toxins by *P. oryzae* in rice seedlings. In metal

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contaminated environment crop production drops due to the reduced rate of photosynthesis caused by irreversible inhibition of extrinsic polypeptides of photosystem II. Besides, there is a reduced rate of thylakoid membrane ion transport in plants exposed to heavy metal stress. The present work aims to study the possible effect of nickel induced protection against blast disease evident from OJIP fluorescence transient as well as other biochemical responses in *Oryza sativa* L. The novelty of the work lies in determining this anti-blast inducing level of Ni (II) without hampering the growth and development of the rice plants significantly unlike similar effects induced by Cd (II) reported by Jali *et al.,* 2019.

Material and Methods

Plant and fungal materials

Dry seeds of *Oryza sativa* L. Var. Naveen were collected from National Rice Research Institute (NRRI), Cuttack, Odisha. Uniform seeds were selected and applied with 0.1% Bavistine for 20 min, then successively washed with 70% alcohol for 30 s and with sterilized distilled water followed by 0.1% mercuric chloride for 5 min. Seeds were incubated overnight inside a growth chamber kept in tightly packed glass jar with ample moisture and kept for germination. The fungus *Pyiricularia oryzae* culture was obtained from Institute of Microbial Technology, Chandigarh, India, with an accession no. 1477. Oat meal Agar/Broth Medium (OMA/OMB) slant and petridish were used for maintenance and inoculums preparation under an aerobic condition at 27±1°C for 14 d in an incubator.

Seedling growth and nickel treatment

Two days old seedlings were moved to Miyamoto's Nutrient solution maintained pH at 5.8 and planted in hydroponic glasses. For seedlings growth, white light (12^{th} photoperiod) (36 Watt; Philips LED) with a photon flux density of 52 μE $m^{-2}s^{-1}$ (PAR) had been irradiated. When plants reach the sampling stage, it was transferred to a greenhouse having regulated climatic conditions required for plant growth. After 10d of seedling growth, plants were treated with Ni, each of 10 ml of varied concentrations (40 μM) and the control seedlings were added with 10 ml of nutrient solution.

Inoculums preparation and inoculation of fungus pathogen

Pure cultures of 14d old *P. oryzae* broth cultures were prepared in OMB medium. The concentration of conidial suspension was adjusted 1×10^5 ml^{-1}conidia using a haemocytometer. For hydroponic experiments 2ml (1×10^5 ml^{-1}) of suspension culture were used. After three days of Ni treatment to the hydroponically cultured plants, fungus pathogen inoculums were sprayed artificially on the wounded leaf surfaces (pricking with sterile pins) using a sprayer on both control and Ni treated plants (40 μM). The inoculated seedlings were incubated at 23°C with > 90% relative humidity (RH) with 12 hr photoperiod for pathogenicity assessment.

Morphological Analysis

A set of 3 seedlings of each treatment were carefully taken out from the hydroponic cups and the root and shoot lengths were measured (in cm) and their mean was calculated. Three replicates of each treatment were washed thoroughly and soaked. Fresh weight (in mg) of root and shoot were recorded using digital balance. There after the seedlings were kept in an oven at 70°C over 3d to get the dry weight (in mg).

Physiological Analysis

Chlorophyll a fluorescence measurement and yield analysis

The chlorophyll *a* fluorescence was measured from the fully open leaves from the growing apex using a Plant Efficiency Analyser (Handy PEA; Hansatech Instruments Ltd., UK). After 10-15 min of dark-adaption with a leaf clip (4 mm diameter), fluorescence rise was recorded by applying continuous actinic red light (685 nm) at an irradiance of 2500 μmol photon/m^2s for 1 s (Strasser and Strasser 1995). The OJIP fluorescence peaks were measured after 50 μs (F_0), 300 μs (F_K), 2 ms (F_J), 30 ms (F_I) and at t_{FM} (F_M=Fp) respectively. The required bioenergetic parameters *viz.*,: maximum quantum yield (F_V/F_M), area under fluorescence, fluorescence at J level (V_J) net rate of PSII closure (M_0), effective antenna size of active RC (Abs/RC), and performance index (PI) were studied to observe the effect of fungus and Ni (II) on rice seedlings.

Biochemical Analysis

The biochemical parameters like chlorophyll, carotenoid, carbohydrate, reducing sugar, protein, proline content were estimated by Arnon (1949), Hedge and Hofreiter (1962), Lowry *et al.,* 1951 and Bates *et al.,* 1973. The activities of antioxidant enzymes like catalase (CAT), guaiacol peroxidase (GPX) and ascorbate peroxidase (APX) were determined by Aebi (1984), Putter (1974) and Nakano and Asada (1981).

Results

Nickel being an essential micronutrient, it has both positive and negative influences on plant. In the present study we used nickel as a model limiting factor to study the antagonistic effect of nickel on fungus pathogen

Pyricularia oryzae and to determine the disease resistance capacity of *Oryza sativa* to blast disease. Thus following morphological, physiological, and biochemical parameters show the growth responses of experimental plant *Oryza sativa* grown hydroponically in controlled conditions under abiotic and biotic co-stress.

Effect of Ni (II) and P. oryzae on growth Parameter

Nickel at higher concentration significantly reduced the length and biomass of root/shoot. By contrast, at lower concentration (40µM) had much higher shoot height and weight of root and shoot than control and fungal infected plants (Table 2). The significant difference in the growth parameters like length, fresh and dry weight of plants were detected amongst the treated plants and plants that were infected with fungus in 10, 20 and 30 d, while the plant heights and dry weights (root and shoot) were significantly more in 30 d plant.

Effect of Ni (II) and *P. oryzae* on Physiological Parameters

Analysis of Chlorophyll a fluorescence transient (O-J-I-P)

There was a marked variation in the curvature of the fluorescence transient in *P*. oryzae infected rice seedlings under Ni (II) treatment over the experimental period. After 10d of treatment, characteristics OJIP rise with clear intermediate peaks (coincides with each other) in the control as well as Ni (40 µM) treated seedlings, were observed. When control compared with fungus infected and fungus with 40 µM Ni (II) treatment (cited as F + Ni (II) (40 µM)) seedlings, a significant change in F_0, F_J, F_I and F_M values was noticed in each concentration. After 20d of treatment, O-J phase did not show any significant variation while J-I and I-P phases demonstrated pronounced difference among the treatments. In a comparison, except for control plants, all other treated plants exhibit a decline in intermediate electron transport and PS I relaxation. After 30 days of treatment, Ni (II) treated plants demonstrated better photosynthetic quenching by fitting OJIP curve exactly with control plants whereas fungus infected rice plants showed high F_0 and low F_M. Interestingly, in combination with Fungus, Ni (40 µM) treated plants overcome the effect of fungus and showed an OJIP curve at par with control (Fig. 1).

Effect on JIP-test parameters

The fluorescence derived parameters showed a decrease in photosynthetic activity and quantum yield (Fv/Fm) in fungus-infected seedlings than control, but in the presence of Ni (II) a gradual recovery in fluorescence signals was observed in infected plants. Similar scores of F_V/F_M, the normalised complimentary area under OJIP curve, RC/ABS and PI values were showed by F+ 40 µM Ni (II) treated seedlings in compared to control in 10 d after treatment. Quantum yield and PI value were observed to become significant at F+ 50 µM Ni (II) after 20 d of treatment (data not shown) whereas F+ 40 µM Ni (II) treated seedlings exhibited the highest area under OJIP transient, PI_{ABS} and RC/ABS value than fungus infected seedlings. Interestingly, F+ 40 µM Ni (II) treated rice seedlings showed enhanced values of F_V/F_M, area, RC / ABS $PI_{\tilde{O}}$ and PI_{ABS} in 30 days than that of fungus infected seedlings (Table 1). PI_{ABS} and $PI_{\tilde{O}}$ parameters showed better performance in co-stressed plants. Throughout the experimental period, infected rice seedlings showed significantly low fluorescence parameters ($F=5.72$ at $P<0.05$).

Effect of Ni (II) and *P. oryzae* on Photosynthetic Pigments

Total chlorophyll content

At 40 µM Ni (II) concentration the chlorophyll content increased by 29% and 22% in 20 and 30d seedlings than control whereas fungus treated seedlings the same were decreased than control by 8%, 50% and 58% for 10, 20 and 30 d respectively. F+40 µM Ni(II) seedlings were showed rise in chlorophyll pigment about 29% and 40% in 10d, 10% and 123% in 20 days and 5% and 156% in 30d than control and fungus treated seedlings respectively. At F+40 µM Ni (II), chlorophyll content raised many folds than other concentrations of fungus treated seedlings (Fig. 3a).

Carotenoid content

Carotenoid increases in 40 µM Ni (II) concentration about 55% than control followed by a fall found in fungus treated plant. There was a significant fall of carotenoid content in fungus infected seedling notice. Thereafter stabilisation of the pigment concentration was observed in F+40 µM Ni (II) treated plants (Fig. 3b).

Effect of Ni (II) and *P. oryzae* on Biochemical Parameters

Reducing sugar

Amount of reducing sugar increased gradually up to 30 µM (data not shown) and decreased significantly from 40 µM Ni (II) onwards. About 6% rise in 10d, 11% rise in 20d and 12% rise in 30d at 30 µM as compare to control. Whereas fungus infected seedlings showed 7% fall in 10d, 8% fall in 20d and 27% fall in 30d from control. About 4.5%, 4.8% and 4.8% increased at F+40 µM Ni (II) treatments as compare to control in 10, 20d and 30 days treated seedlings. But from fungus infected seedlings, F+40 µM Ni (II) showed 13%, 29% and 44%

Table 1: The data showing the changes in quantum yield specific energy flux and performance parameters in *O. sativa* (Naveen) under co-stress of *P. oryzae* and Ni (II).

10 d after treatment				
Fluorescence Parameters	**Control (NC)**	**40 µM Ni**	**Fungus infected (PC)**	**F + 40 µM Ni**
Fv/Fm	0.818 ± 0.001	0.8115 ± 0.002	0.7948* ±0.003	0.8118 ± 0.001
Area	20056 ± 385	21031 ± 1555	13295* ± 359	19887 ± 393
RC / ABS	0.6916 ± 0.009	0.7113 ± 0.024	0.4925* ± 0.016	0.6811 ± 0.009
PI_{ψ}	0.4525±0.002	0.4408 ± 0.008	0.3551* ± 0.010	0.4475 ± 0.006
PI_{ABS}	1.4127±0.025	1.3545 ± 0.066	0.6907*±0.055	0.98* ± 0.029
20 d after treatment				
Fv/Fm	0.8211 ± 0.003	0.7951 ± 0.005	0.7652* ± 0.006	0.8046 ± 0.002
Area (N)	17068 ± 766	11549 ± 891	9096* ± 578	14503 ± 298
RC / ABS	0.6491 ± 0.018	0.5223 ± 0.031	0.4642* ± 0.035	0.6077 ± 0.006
PI_{ψ}	0.462 ± 0.009	0.4055 ± 0.020	0.3266* ± 0.027	0.4231 ±.006
PI_{ABS}	1.4018 ± 0.098	0.8719* ± 0,111	0.5439* ±0.110	1.06 ± 0.032
30 d after treatment				
Fv/Fm	0.8171 ± 0.002	0.8176 ± 0.003	0.7476* ± 0.007	0.7896 ± 0.008
Area (N)	18083 ± 946	16160 ± 866	8695* ± 511	16877 ± 591
RC / ABS	0.6846 ± 0.022	0.6741±0.020	0.3965* ± 0.016	0.5338 ± 0.028
PI_{ψ}	0.4843 ± 0.008	0.466±0.009	0.2524* ± 0.024	0.4724 ± 0.026
PI_{ABS}	1.5009 ± 0.099	1.4172±0.070	0.327* ± 0.069	1.1196 ± 0.121

Note. Values in the table are mean ± standard deviation of seven replicates. *indicates significant differences between treatments at P d" 0.05 (5% level of significance) for each parameters as per the Fishers F-test.
Abbreviations: Fv/Fm - Maximal quantum yield of primary PS II photochemistry; N- Turnover number, i.e. number of Q_A reduction events between time t_0 and t_{FM}; RC/ABS - Active RCs on absorption basis; PI_j - Performance index of primary photochemistry; PI_{ABS} -Performance index for energy conservation from photons absorbed by PS II antenna to the reduction of Q_B, PC- Positive Control, NC- Negative Control.

highest rise in 10,20 and 30d treated seedlings (Fig. 3c).

Protein content

Ni (II) at 20 µM concentration increased protein content in small amount (1%) than control and significantly reduced from 30 µM onwards in all 10, 20 and 30 days treated seedlings. And even fungus infected seedlings showed very low impact about 1% and 3% decrease from control in 10 and 20 days treated seedlings on protein contents but rose to 8% fall from control in 30 days. And though F+40 µM Ni (II) treated seedlings showed fall in protein content than control but raised about 1% in 10 days, 2% in 20 days and 6% in 30 days compared to fungus infected seedlings (Fig. 4a).

Proline content

Proline content rose with increasing Ni (II) concentration and highest increase showed at 50 µM Ni (II) in all 10, 20 and 30 days treated seedlings. But subsequent days of exposure showed decrease in proline content from 90% in 10 days to 77% in 30 days at 40 µM Ni (II) than that of control. Similar trend also found in fungus treated seedlings from 10 days to 30 days and the percentage of fall is from 46% in 10 days to 40% in 30 days than control. Whereas in F+40 µM Ni (II), in 10 days about 13% decrease in proline content found than control but raised to 9% in 20 days and 31% in 30 days than control, signals the effect of both Ni (II) on fungus. But compared to fungus infected seedlings, F+40 µM Ni (II) showed gradual fall in proline content about 41% in 10 days, 22% in 20 days and 6% in 30 days treated seedlings (Fig. 3d).

Antioxidative enzyme activity

The activity of Catalase in fungus infected seedlings showed significant decrease in trend where it was decreased to 8% in 20d and 22% in 30 d as compare to control. F + 40 µM Ni (II) treatment showed significant rise in activity compared to both fungus infected and control plants. The antioxidative activity of Guaiacol peroxidase (GPX) increased in all treatments but vary in percentage of increase and according to days of exposure. 40 µM Ni (II) concentration showed highest increase in percentage of GPX compare to control. Fungus infected seedlings showed increasing trend of 175% in 10 d, 130% in 20 d and 66% in 30 d treatment as

Table 2: Different morphological and growth parameters of *O. sativa* (var. Naveen) under Ni (II) and *P. oryzae* infection stress. Values are mean ± Standard Deviation of five replicates.

Treatment Interval	Growth Parameters	Control	40 µM Ni	Fungus infected	F + 40 µM Ni
10 d	RFW (mg)	33.7 ± 0.23	55.4 ± 0.18	33.5 ± 0.19	52.6 ± 0.41
	RDW (mg)	4.8 ± 0.32	7.9 ± 0.34	4.4 ± 0.43	7.4 ± 0.41
	SFW (mg)	48.6 ± 0.23	74.5 ± 0.14	50.4 ± 0.11	73.2 ± 0.40
	SDW (mg)	14.2 ± 0.18	20.9 ± 0.15	12.6 ± 0.22	19.7 ± 0.41
	RL (cm)	9.2 ± 0.12	11.3 ± 0.17	7.9 ± 0.26	10.5 ± 0.38
	SL (cm)	11.9 ± 0.23	12.9 ± 0.31	9.0 ± 0.07	11.3 ± 0.35
20 d	RFW (mg)	41.3 ± 0.27	62.3 ± 0.23	37.8 ± 0.39	61.2 ± 0.25
	RDW (mg)	6.3 ± 0.19	10.7 ± 0.26	4.1 ± 0.27	9.3 ± 0.33
	SFW (mg)	51.9 ± 0.43	83.8 ± 0.42	49.7 ± 0.44	76.3 ± 0.16
	SDW (mg)	16.3 ± 0.17	21.9 ± 0.43	11.6 ± 0.34	20.8 ± 0.15
	RL (cm)	11.0 ± 0.22	13.9 ± 0.15	8.8 ± 0.28	11.9 ± 26.0
	SL (cm)	12.2 ± 0.40	12.6 ± 0.17	11± 0.39	11.6 ± 0.14
30 d	RFW (mg)	47.2 ± 0.27	65.6 ± 0.07	40.2 ± 0.37	63.2 ± 0.35
	RDW (mg)	8.2 ± 0.33	12.6 ± 0.31	3.6 ± 0.43	9.7 ± 0.24
	SFW (mg)	60.1 ± 0.34	94.6 ± 0.24	49.3 ± 0.25	82.3 ± 0.19
	SDW (mg)	18.2 ± 0.29	27.2 ± 0.43	10.4 ± 0.37	21.7 ± 0.21
	RL (cm)	13.0 ± 0.17	16.9 ± 0.41	9.3 ± 0.22	12.9 ± 0.43
	SL (cm)	13.6 ± 0.23	14.6 ± 0.37	11 ± 0.24	14 ± 0.012

Abbreviations RFW- Root Fresh Weight, RDW- Root Dry Weight, SFW- Shoot Fresh Weight, SDW- Shoot Dry Weight, RL- Root Length, SL- Shoot Length.

compared to control. Whereas F + 40 µM Ni (II) treatment 212% and 13% rise in 10 days, 180% and 21% rise in 20 days & 166% and 60% rise in 30 days showed as compared to control and fungus infected seedlings respectively. Unlike GPX activity, APX activity also increased in 20 d and 30 d in F + 40 µM Ni (II) treatments as compared to control and fungus infected seedlings. There were 126% and 61% rise in 10 days, 118% and 52% rise in 20 days & 117% and 54% rise in 30 days were noticed against control and fungus infected seedlings respectively (Fig. 4b, c. d).

Discussion

The rice is most abundantly cultivated agriculturally important plant and is the prime food in highly populous country like India. In the recent past, to get disease resistant rice variety, the national as well as international institutes put their maximum effort in biotechnological innovations in producing high yield and resistant variety. The pros and cons of GMOs are under debate among the scientific communities. Nickel deficiency causes leaf chlorosis along with necrotic leaf tips whereas bioaccumulation of nickel in higher concentrations severely retards germination. Shoot and root growth, induce leaf spotting, and Fe deficiency etc. leads to chlorosis and foliar necrosis. Excess Ni also affects urease activity, inhibits photosynthesis and transpiration, and causes ultrastructural modifications (Brown *et al.*, 1987).

Synergistic effect of Ni (II) and *P. oryzae* on growth parameters

In the present experiment, 40 µM Ni (II) concentration in *Oryza sativa* L. seedlings showed highest growth than control and 50 µM was identified as LC_{50} and at 100 µM about 80% of seedlings died (data not shown). The effect of nickel on growth of *Oryza sativa* L. seedlings decreased with elevated concentrations of Ni as compared to control (Bhardwaj *et al.*, 2007). In *Brassica juncea* total biomass production increases up to 50 µM and a prominent decrease was found at 100 µM due to decline in water content, transpiration rate and stomatal conductance (Alam *et al.*, 2007).

Fungus has negative impact on rice seedlings and possessed significant reduction in growth of root-shoot length and root-shoot biomass. In presence of Ni (II), fungus pathogenicity decreased towards rice seedlings and growth was almost similar to that of control. From present study application of 30 to 40 µM Ni (II) concentrations to fungus infected seedlings showed positive influences and provide resistance to *O. sativa* L. seedlings (Fig. 2). It was also found that Ni (II) possesses more positive effect in shoot length as compare to root length in presence of *P. oryzae* which may be because of bio-accumulation of Ni (II) in roots increase

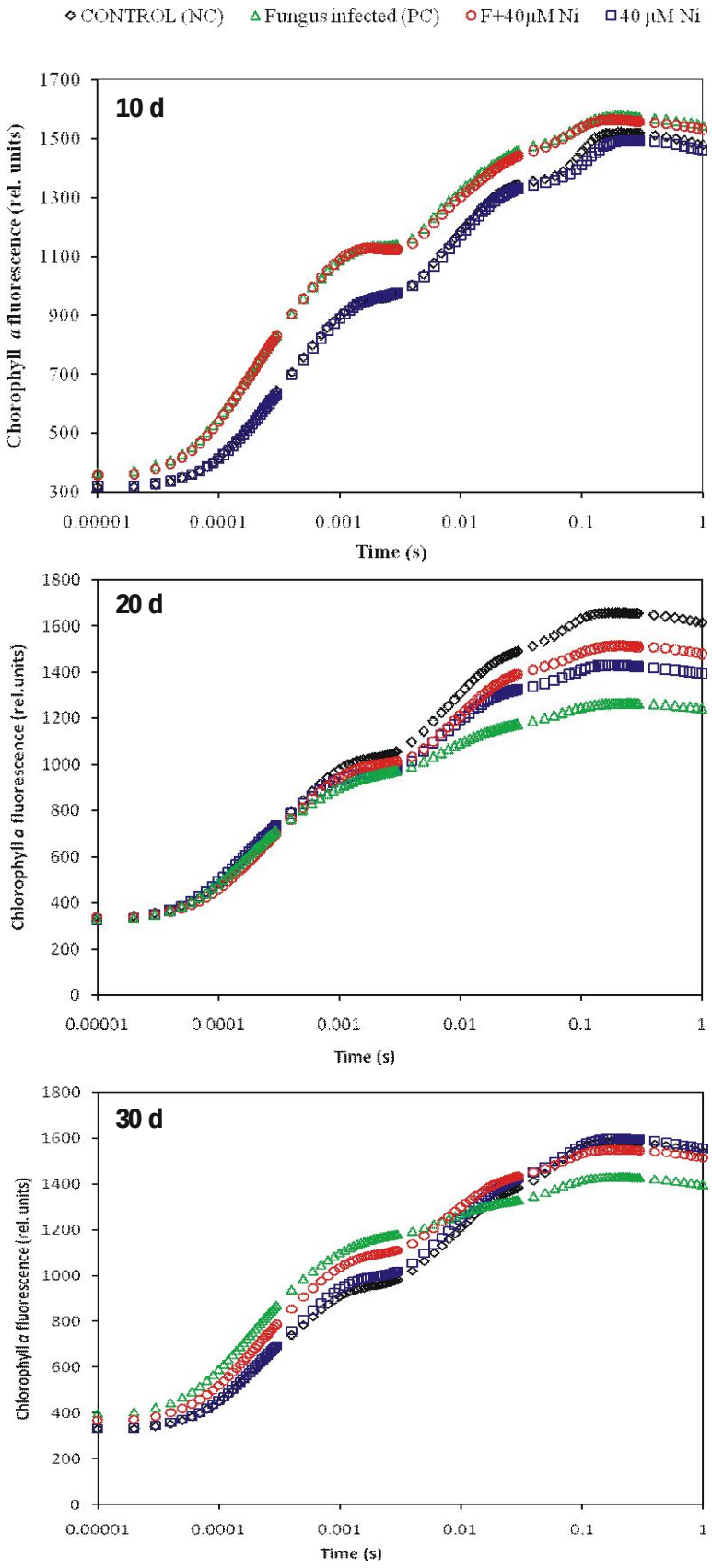

Fig. 1: Effect of Ni (II) and *P. oryzae* on fast polyphasic Chl *a* fluorescenc rise (OJIP) in *O. sativa* L. (var. Naveen) after10, 20 and 30 d of treatment.

in root dry matter found than that of control seedlings. It was further noticed that with increasing in days of exposure Ni (II) found friendlier for rice seedlings in presence of *P. oryzae*.

Synergistic effect of Ni (II) and *P. oryzae* on Photosynthetic Pigment and Chlorophyll fluorescence

Changes in photosynthetic pigments

Reduction in photosynthetic activity in fungus-infected seedlings might be due to the release of fungal toxins that inhibit the photosynthesis and/or destruct the photosynthetic apparatus. But the photosynthetic activity of fungus treated seedlings gets increased in the presence of Ni (II). In co-stress condition plants showed enhancement in Chl *a*, Chl *b* and carotenoid levels in lower concentrations. Whereas the fall of carotenid content in F+40 µM Ni (II) treatment may be because of an increase in chlorophyll content more than control. The F + Ni (II) plants were observed to have enhanced photosynthetic response than the fungus only. This result was in agreement with the findings reported in *Brassica napus* infected with pathogen and Cd (Larsen *et al.*, 1998).

Changes in the fluorescence peaks of OJIP transient

OJIP fluorescence transient is the fluorescence emission released primarily from PS II and represents various PS II reactions (Tsimilli-Michael and Strasser 2008). The O - step of the transient refers to the fluorescence released from the photosystems when the leaves are sufficiently dark-adapted and it is expected that all active photosystems are in an open state (Strasser *et al.*, 2000). The OJIP transient is considered as the fluorescence rise when there is no energy transfers between the PS II units as if they exists independently. Thus, the fluorescence recorded at different times of the transient is an indication of the behaviour of PS II in response saturating pulse.

There was significant change in the shape of the transient on the exposure of the plants to graded Nickel (II) concentrations in combination with or without biotic stress. After 10 days, F + 40 µM Ni(II) seedlings showed similar OJIP transient as fungus-

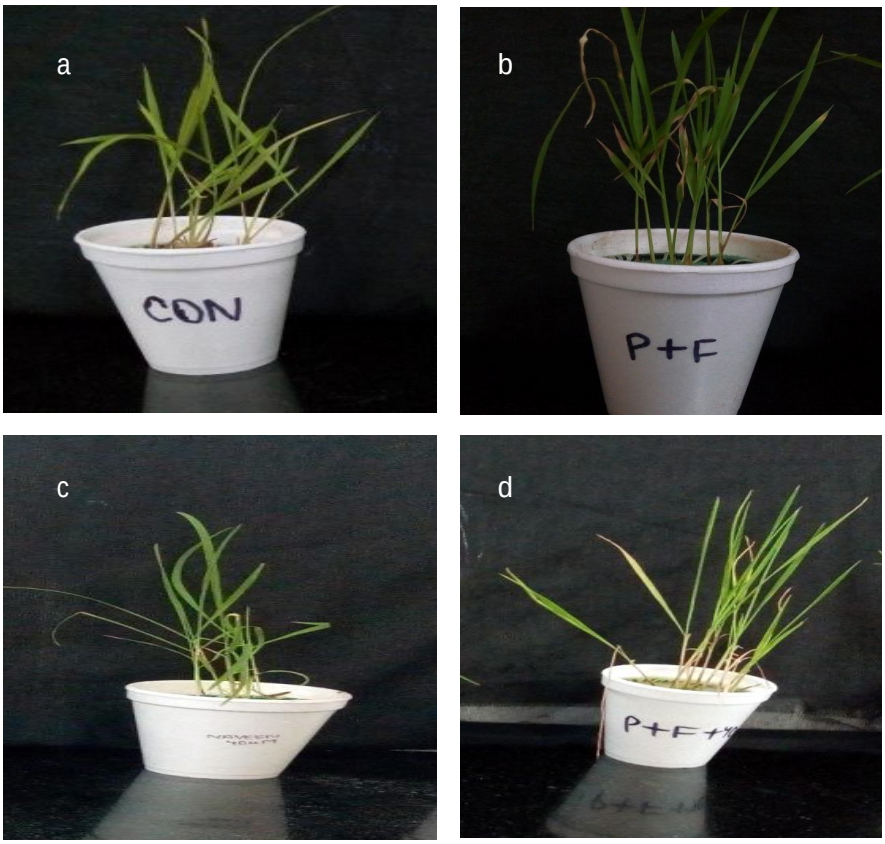

Fig. 2: Photographs showing (a) control without treatment (CON), (b) Fungus infected plants (P+F) (c) 40 uM Ni (II) treated plants, and (d) fungus infected with 40 µM Ni (II) treated (P+F+40) *Oryza sativa* L. (Var: Naveen).

infected seedlings whereas 40 µM Ni (II) treated plants showed similar transient patterns as those of control plants indicating Nickel as a micronutrient rather than a stressor. However, after 20 days of treatment, almost all the treated plants demonstrated a significant decrease in J-I and I-P transient than control plants. Maximal fluorescence F_M indicates that after 20 d of treatment, *P. oryzae* infection in rice reduces photochemical quenching significantly. Interestingly, 30 d after treatments, F + 40 µM Ni (II) treated plants exhibited best fit OJIP fluorescence transient with control. But fungus-infected plants showed a rise in F_0 and decline in F_I and F_M which indicated several RCs in closed state *i.e.* problem in water splitting activity lead to a decrease in the reduction of P_{680}^+. No further fluorescence enhancement beyond F_J indicates complete inhibition of Q_A- Q_B electron flow which was observed for fungus infected rice plants. Thus, at I-P transient phase, the Ni (II) negatively influences the fungus growth. The O-J-I-P fluorescence transient showed increased photosynthetic activity in co-stress of fungus and Ni (II) than positive (with fungus + Ni(II)) as well as the negative control (with Ni(II) only).

Change in fluorescence derived parameters of JIP-test

Chlorophyll a fluorescence, though corresponding to a very small fraction of the dissipated energy from the photosynthetic apparatus, is widely accepted to provide an access to understand its structure and function. Chl *a* fluorescence is signature of photosynthesis, more specifically on PS II when fluorescence kinetics, spectra, lifetime and derived parameters are analysed (Stribet and Govindjee 2011). In the present context, various quantum yield parameters and stress indicating parameters were taken up for prediction of PS II behaviour under the combination of Ni (II) and fungus induced stress as well as to analyse the efficiency of Ni (II) in the rice plant to resist blast. The performance indicating parameters like F_V/F_M or jP_0, jE_0 and y_0 showed an increase under co-stress condition which can be correlated with biochemical responses.

It was observed that under the influence of Nickel (II), the rate of PS II closure increased, which could be significant in all the treatments. The higher values of M_0 jn 40 µM Ni(II) prove that there were some disturbances on the OEC leading to limitation of PS II function. Thus, it can be inferred that Ni (II) stress not only caused donor limitation of PS II, but also forced an acceptor limitation through enhanced single turn over events of Q_A. The reduced end reduction (RE^1) also supports this conclusion (results not shown). Higher single turn over event of Q_A

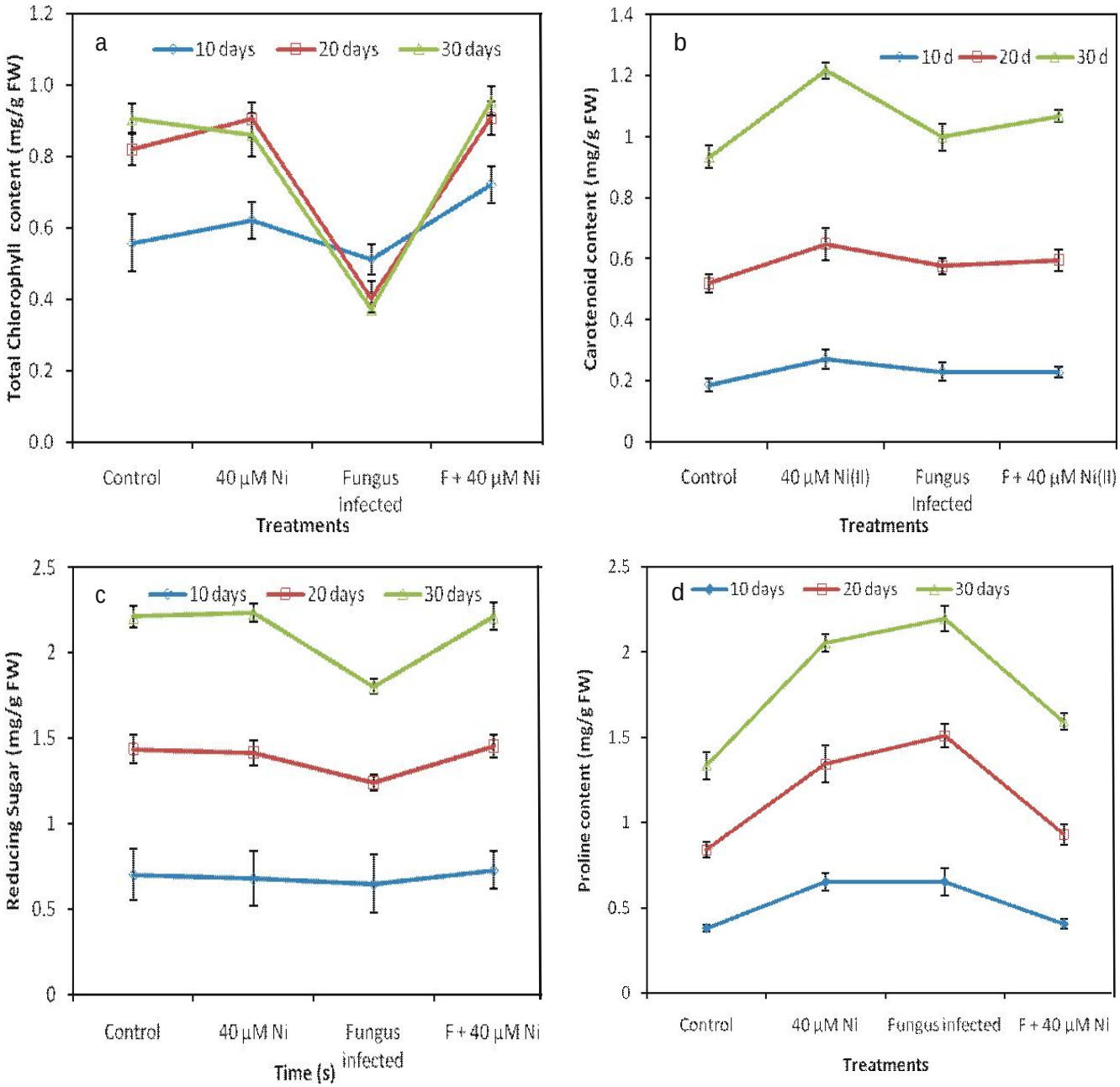

Fig. 3: Effect of Ni (II) and *P. Oryzae* infection on (a) total chlorophyll content, (b) carotenoid content, (c) reducing sugar and (d) proine content of *O. sativa* (var. Naveen) after10, 20 and 30 d of treatment.

electron movement beyond PQ pool resulting in enhanced in RE in higher Ni concentrations with co-stress conditions and thereby the photosynthetic performance.

A lower absorption per RC is an indicator of better performance with growth-supporting levels of Ni induced resistance against rice blast and could be well correlated with net photosynthesis. Increased ABS/RC values at the growth retarding salt concentration in the present case is since Ni stress caused activation of the part of RCs resulting in proportionately more light absorption than clear reduction therefore, the nonphotosynthetic energy dissipation. This is also supported by an enhanced dissipation per RC in diseased rice plants and a proportionate reduction of active RCs. It may be concluded that Ni(II)- stress inhibited PS II reaction centres causing shrinkage of active PS II, which could be responsible for reduced photosynthetic activity as this could not take part in photosynthetic electron transport.

The present study, therefore, used the performance indices PI_{δ} and PI_{ABS} for effective analysis of plant performance. It also takes into account the nonphotochemical events to make it a holistic performance indicator. The reduced rate of performance $\geq$ 40 µM Ni (II) in diseased rice showed that the species is not a

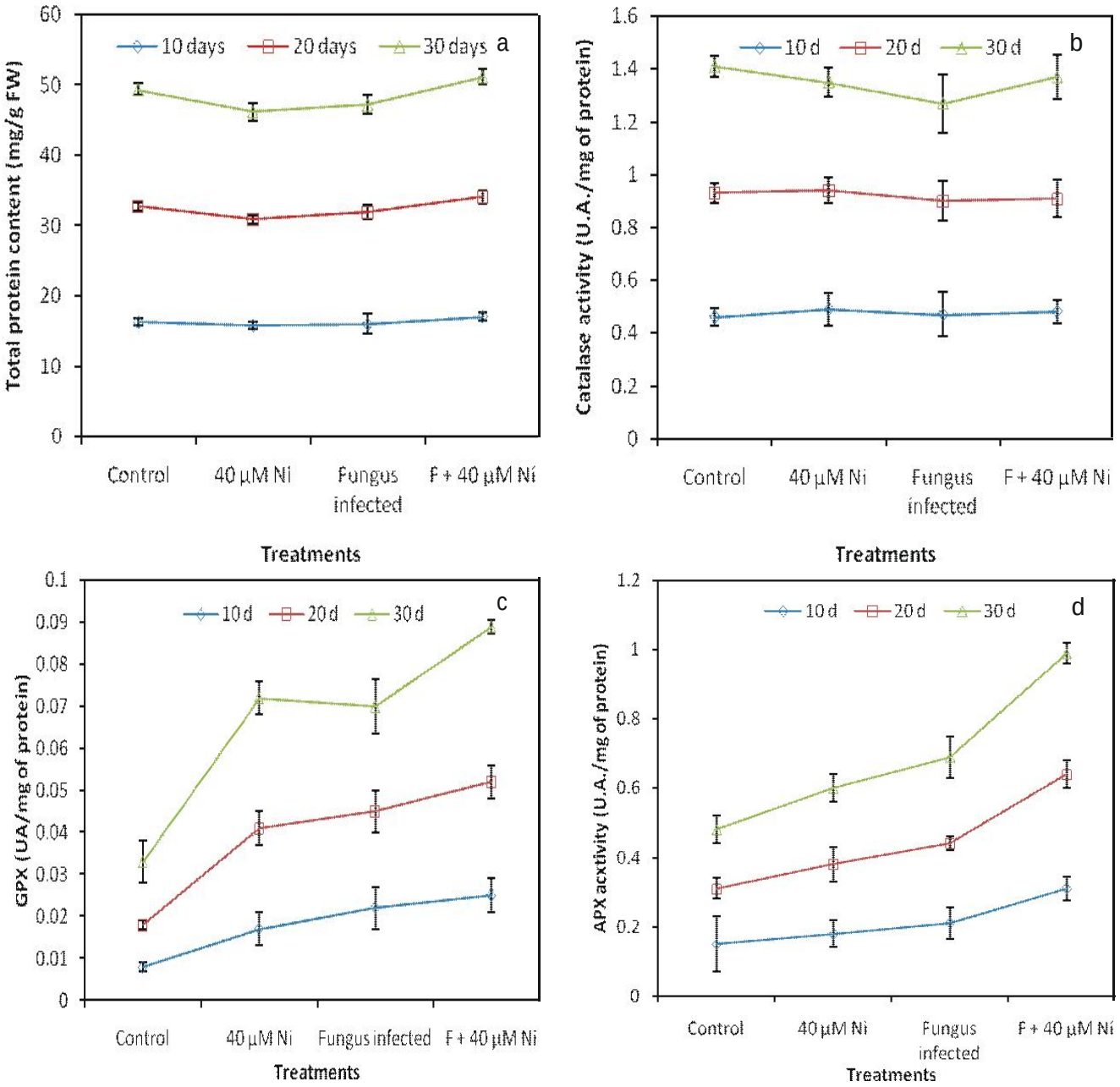

Fig. 4: Effect of Ni (II) and *P. Oryzae* infection on (a) total protein content, (b) Catalase activity, (c) Guaicol Peroxidase activity (GPX) and (d) Ascorbate Peroxidase activity (APX) of *O. sativa* (var. Naveen) after10, 20 and 30 d of treatment.

good candidate for consideration rather introduction in areas of low doses. The 40 µM concentrations of Ni (II) be a good candidate to induce blast resistance in rice..

Changes in Biochemical Parameters

Fungus infected seedlings showed significant reduction in both carbohydrate and reducing sugar content, that might be because of breakdown of polysaccharides by toxic proteins released by fungus *P. oryzae*. The protein contents decreased significantly in diseased rice plants with Ni (II) application. But in co-stress condition (F+40 µM Ni (II)) the protein content increased with increasing days of exposure. Thus nickel has adverse impact on protein content but when nickel applied to fungus infected seedlings; it increases the protein content than fungus infected seedlings. The inhibition of protein accumulation might be due to blockage in translation mechanism after exposure to fungus and Ni stress which common in both biotic and abiotic stress. The proline is a stress induction marker. Seedlings exposed to *P. oryzae* and Ni showed increase in proline content but percentage of increase in Ni (II) treated seedlings rise gradually. Whereas in co-stress the proline content decreased gradually up to F +40 µM Ni (II) treatment, which indicates reduction of stress. Thus Ni (II)

compensates the infection of fungus that results in decrease in proline content.

Changes in Enzyme Activity

Plant possesses a number of antioxidative enzymes to protect against oxidative damage induced by biotic and abiotic stress. The present investigation showed rise in APX, CAT and GPX activity in F + 40 µM Ni (II) concentrations and act as markers under stress conditions. The enhancement in Ascorbate Peroxidase (APX) activity under Ni as well as fungus stress indicates its role in detoxification of H_2O_2 in *Z. xanthoxylon*. These antioxidative enzyme activities were significantly enhanced in co-stress than fungus infected rice seedlings (Fig. 4).

Catalase (CAT) scavenges free radicals in defense against oxidative damage by spliting down H_2O_2 (Mittler 2002; Patra *et al.,* 2018a, b; Patra *et al.,* 2019; Patra *et al.,* 2020). Alterations in CAT activities were observed in differential regulation induced by nickel stress (Gajewska and Sk³odowska 2007). The present findings showed increased CAT activities at concentrations up to 40 µM Ni (II) in fungus infected rice seedlings exhibiting defensive mechanisms against nickel stress.

GPX is a metalloenzymes provides cell defense against oxidative stress induced by environmental stresses *viz.,* chilling, air pollution, heavy metals, salts, pathogen attack and UV radiation (Passardi *et al.,* 2005). Unlike APX and CAT, F + 40 µM Ni (II) treatments corroborates significant rise in GPX as compared to control and fungus infected seedlings indicating balanced metabolic activity against both the stressors.

At low concentrations nickel promotes the activities of the various enzymes including IAA oxidase, ascorbate oxidase, catalase and peroxidase. The physiological and enzymatic activity of nickel depends upon the variety of plant species under investigation. The concentration exceeding the threshold concentration reduces the enzymatic and other physiological activities. In this present experiment, both *P. oryzae* and Ni (II) induced the activities of CAT, GPX and APX but in small quantity whereas upon exposure of *P. oryzae* with Ni (II), CAT, GPX and APX activity increased with increasing days of exposure than fungus infected seedlings. *i.e.* F + Ni (II) 40µM treated seedlings showed stronger superoxide and hydrogen peroxide scavenging system than fungus infected seedlings.

Conclusion

Being a micronutrient, Ni (II) always shows a positive influence on plants especially to crop and cereals. Not only in enzyme activity but also morphological, biochemical and physiological activity induction Ni (II) plays an important role in growth-producing factors, unlike other heavy metals as evidence from the present investigation. Thus, according to the present study, at low doses, Ni (II) increases the growth as well as antifungal activity of *O. Sativa* L. seedlings against blast causing fungus *P. oryzae*. The hydroponically cultured rice variety showed the highest growth at 40 µM of Ni (II) concentration which showed growth in morphology, photosynthetic activity, and biochemical constituent related like chlorophyll, carotenoid, carbohydrate, reducing sugar, protein and proline content and antioxidative enzymes.

During co-stress of *P. oryzae* Cav. with Ni (II) in 10 d, 20 d, and 30 d experiment showed at F + 40 µM Ni(II) treatment seedlings showed highest morphological and biochemical development than other treatments of Ni(II) compared with fungus-infected seedlings. The fluorescence peaks and area under O-J-I-P curve suggest there was a growth retarding effect of fungus in *O. sativa* seedlings whereas at 40 µM Ni(II) concentrations exhibited a better rate of electron transport per active reaction centre cores. The quantum yield parameters F_V/F_M and performance indices also showed similar trends. At co-stress conditions showed donor limitation in primary photochemistry up to 20 days of exposure and acceptor (electron) limitation beyond $Q_{A.}$ A positive correlation between chlorophyll content and photosynthetic response in terms of OJIP fluorescence transient was observed. Thus, there might be some down regulations in 40 µM Ni(II) application that might be a release of fungus-resistant protein or enzymes resulted highest growth as well as put impact on fungus *P. oryzae* Cav. and plants possess systematic acquired resistance to fungus *P. oryzae* Cav. causing rice blast disease in *O. sativa* L. Thus, in the present study, Ni (II) showed an antagonistic effect on *P. oryzae* Cav. as well as act as a micronutrient supplement at 40 µM levels. In the present investigation, it may be hypothesized that Ni (II) at moderate concentrations induces a stress response in rice plant thereby inhibiting an antagonistic effect of *P. oryzae* causing blast disease in rice.

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Conflict of interest

The authors declare that they have no conflict of interest

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