Effect of Lead levels on germination attributes and biochemical parameters in rice (*Oryza sativa* L.)

काशी हिन्दू विश्वविद्यालय BANARAS HINDU UNIVERSITY

THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE AWARD OF THE DEGREE OF

Masters of Science (Agriculture)
In
Plant Physiology

Supervisor
Dr. Savita Jangde

Submitted by
Abhilasha Choudhary

DEPARTMENT OF PLANT PHYSIOLOGY
INSTITUTE OF AGRICULTURAL SCIENCES
BANARAS HINDU UNIVERSITY
VARANASI (U.P.) – 221005
INDIA

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काशी हिन्दू विश्वविद्यालय BANARAS HINDU UNIVERSITY

Dr. Savita Jangde
Professor
Department of Plant Physiology
Institute of Agricultural Sciences,
Banaras Hindu University,
Varanasi – 221005
Phone No.- +91 7617018105
E-mail- savi.bhu31@gmail.com

Ref. No.:…….. **Date:……**

CERTIFICATE

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Banaras Hindu University
Varanasi – 221005(INDIA)

Through: The Head, Department of Plant Physiology, Institute of Agricultural Sciences, B.H.U, Varanasi.

Dear Sir,

I have great pleasure in forwarding the thesis entitled **"Effect of Lead levels on germination attributes and biochemical parameters in rice (*Oryza sativa* L.)"** submitted by **MS. Abhilasha Choudhary.**, **I.D. No. 18412PLP019**, in partial fulfillment of the requirements for the degree of **Master of Science (Agriculture) in Plant Physiology** in the Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P).

I certify that the entire work reported herein, was planned and carried out by the candidate under my guidance and supervision and to the best of my knowledge and belief, the data presented in the thesis are genuine and original.

Thanking you.

Forwarded by

Dr. Savita Jangde

(Head of Department) (Supervisor)

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काशी हिन्दू विश्वविद्यालय BANARAS HINDU UNIVERSITY

By

Abhilasha Choudhary

Department of Plant physiology
Institute of Agricultural Sciences
Banaras Hindu University
Varanasi-221005

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THESIS APPROVED BY ADVISORY COMMITTEE

Chairman : **Dr. Savita Jangde**
Professor
Department of Plant Physiology
Institute of Agricultural Sciences,
B.H.U., Varanasi.

Member : **Dr. Vijai. P**
Professor
Department of Plant Physiology
Institute of Agricultural Sciences
B.H.U., Varanasi

Member : **Dr. Kalyan Barman**
Assistant Professor
Department of Horticulture
Institute of Agricultural Sciences,
B.H.U., Varanasi.

External Examiner:

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B.H.U. Varanasi.
, 2022 **(*Abhilasha Choudhary*)**

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ABBREVIATIONS

Abbreviations	Full form
%	Per cent
&	And
As	Arsenic
USDA	United State Department of Agriculture
FAO	Food and Agriculture Organization
DAS	Days after sowing
et al.	Co- authors (et allii)
etc	Etcetera
SEm±	Standard error of mean
g	Gram
Hrs	Hours
i.e.	That is (id est.)
Kg	Kilogram
Kg ha^{-1}	Killogram per hectare
M	Meter
Mg	Milligram
mha.	Million hectare
ml l^{-1}	Milli litre per litre
mm	Milimeter
CRD	Complete Randomized Design
viz.	Videlicet; read as namely
Pd	Lead
ROS	Reactive oxygen species
ATSDR	Agency for Toxic Substances and Disease Registry
Cd	Cadmium
SOD	Auperoxide dismutase
APX	Ascorbate peroxidase
DHAR	Dehydroascorbate reductase
MBP	Myelin basic protein
CDPK	Calcium-dependent protein kinase
MAP	Mitogen-activated protein
TBARS	Thiobarbituric acid reacting substances
ETC	Electron transport chain
POD	Peroxidase

CHAPTER-I

INTRODUCTION

Rice (*Oryza sativa*) is one of the most important cereal crops, providing food for nearly a half of the world population (Panich-pat and Srinives, 2009) and contributing with one fifth of the calories consumed by human's worldwide (Welch and Graham, 2005). About 90 percent of the total rice is cultivated in Asia (FAO, 2019). Rice is the most important food crop of India; not only because of its local consumption but also in view of large exports (USDA, 2020). Globally, rice is cultivated on 162.9 m ha with the annual production of around 508.7 m tones (USDA, 2020). India is the second largest producer of rice after China. In India, rice ranks first among all crops occupying 43.79 m ha area and production of 116.42 m tons of rice with average productivity of 2.65 t ha^{-1} (Agricultural Statistics at a Glance, 2019). Uttar Pradesh is the second largest rice growing state after West Bengal in the country. It is grown in an area of about 5.75 million hectares with the production of 15.54 million tones and productivity of 2.70 tones per hectare (Agricultural Statistics at a Glance, 2019). Rice occupies a pivotal place in Indian agriculture and it is grown under diverse ecologies throughout the country. Rice is highly nutritive crop contains carbohydrate (74.8 %), protein (8.4 %), fat (2.6 %), minerals (phosphorus, calcium, iron, etc.), amino acids, thiamine, riboflavin, niacin, pigments and dietary fibre (Kumar and Ladha, 2011).

Plants absorb many minerals from the soil, some of which are known to have no biological function and some to be toxic at low concentrations. As plants form the basis of the food chain, some fears have been increased about the possibility of toxic amounts of certain minerals being transported from plants to higher layers of the food chain. Therefore, special attention has been paid to the mechanisms of absorption and biotransformation in plants, as well as its role in bio-deposition and its influence on consumers, especially humans (Peralta-Videa *et al.*, 2009). Heavy metal contamination has devastating effects on plant productivity and threatens human and animal health

(Lamhamdi *et al.*, 2011). Plants are in the target of a wide extent of pollutants that have different concentrations, types and toxic effects. The plant system is exposed to such pollutants mainly in the soil (Arshad *et al.*, 2008) or atmosphere (Pourrut *et al.*, 2011; Uzu *et al.*, 2010).

Among different heavy metals, lead (Pb), is the second most harmful pollutant after arsenic and recently listed as "the chemical of great concern" according to the new European REACH regulations (Pourrut *et al.,* 2011). It severely affects normal plant metabolism, morph-physiological features and crop growth and productivity (Sharma and Dubey, 2005; Ashraf *et al*., 2015). It often leads to diminished growth, deformation of cellular structures, ion homeostasis, reductions in chlorophyll biosynthesis, hormonal imbalance and induce over-production of reactive oxygen species (ROS) in plants (Shahid *et al*., 2011; Kumar *et al*., 2012). Pb, being non-redox metal, cause ROS production that led to oxidative stress within plant cells (Singh *et al*., 2010). Once produced, these ROS readily attacks to biological structures and biomolecules and results in metabolic dysfunction (Clemens, 2006).

It is one of the most widely and regularly released heavy metals found in various forms in natural resources all over the world (Divya *et al.*, 2015; LeBrón *et al.*, 2019; Lone *et al.*, 2006) like mining and smelting of lead-ores, burning of coal, effluents from storage battery industries, automobile exhausts, metal plating and finishing operations, fertilizers, pesticides and from additives in pigments and gasoline (Pourrut *et al*., 2011). Lead (Pb) is commonly spread throughout the environment and reveals a comparatively high reactivity to plant cells (Awan *et al*., 2015). Its higher levels in the environment are not only toxic to human beings but also harmful for plantsas well as soil microbes and can cause plant death (Grover *et al.*, 2010; Pourrut *et al.*, 2011; Shahid *et al.*, 2011). Contamination of heavy metals in India has been observed across the nation. Nearly 718 districts have contaminated groundwater with arsenic, cadmium, chromium and lead (Mohan, 2018).

Lead is being added and accumulating profoundly in the soil through anthropogenic activities (Alloway, 2013; Mulligan *et al.,* 2001). Its eminency results

from its current and previous sources and its persistency in the soil (Andra *et al.,* 2009; Punamiya *et al.,* 2010). Agency for Toxic Substances and Disease Registry (ATSDR 2003) ranked lead as the second most harmful element due to its occurrence, toxicity and exposure potential, after arsenic. Being a toxic substance, and having high transfer rates (from soil to plant), it is therefore studied broadly especially in context to food safety, quality and biotesting purposes (Uzu *et al*., 2009). It adversely affects plant's morpho-physiological and biochemical processes such as seed germination and seedling growth, plant phenology, and root/shoot ratio; disrupts cell membrane permeability, photosynthesis, plant respiratory processes, chlorophyll contents, chloroplastic lamellar organization and cell division and cause growth and developmental abnormalities as well as ultrastructure changes (Dogan *et al.,* 2009; Ling and Hong, 2009; Gupta *et al.,* 2009, 2010; Maestri *et al*., 2010). It further results in ROS production such as superoxide radicals (O^{-2}), hydroxyl radicals (OH) and hydrogen peroxide (H_2O_2) that react with micro and macro cellular organelles to cause cell damage (Reddy *et al.,* 2005). However, its effectiveness depends on stress intensity and duration, plant stage of stress exposure, lead concentration and its bioavailability in plant organs. Plants have their own metal uptake, accumulation, translocation, detoxification, excretion, and compartmentalization mechanisms to respond to lead toxicity (Jiang and Liu, 2010). There is a wide range of genotypic variation existing among rice plants regarding uptake, translocation and accumulation of Pb (Cheng *et al*., 2006).

Different rice varieties having high yield potential and less accumulation/uptake of lead to be evaluated under Varanasi conditions. Standardizing location specific varieties under lead toxicity could result in significant improvement in growth and yield of rice. Seed germination and early seedling growth are critical and complex physiological processes in plants that are highly sensitive to lead stress. The lead induced inhibitory effects have been well documented in many plant species, including rice (Kaya *et al*. 2019; Xiong *et al*., 2021; Lamhamdi *et al*., 2011 and Wang *et al.,* 2020).

The adoption of rice under lead toxicity so far has been very limited and there are not many studies on effect of lead nitrate on rice cultivars under such situations. Additional investigation is required in this issue about the physiology and anatomy of rice

exposed to lead (Pb). Thus in context of the above information, an experiment entitled **"Effect of Lead Levels on Germination attributes and Biochemical Parameters in Rice (*Oryza sativa* L.)"** was conducted during *kharif* season 2019 at Department of Plant physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh with the following objectives:

1. To evaluate the effect of lead levels on germination and growth parameters in rice seedlings.

2. To analyse the effect of lead levels on biochemical parameters in rice seedlings

CHAPTER-II

REVIEW OF LITERATURE

A review of literature on important aspects pertaining to the present study entitled "Effect of Lead Levels on Germination attributes and Biochemical Parameters in Rice (*Oryza sativa* L.)" has been presented in this chapter.

2.1 Effect of heavy metal stress on crops

Presence of copious amount of HMs adversely affects the accumulation and transportation of essential elements in plants that ultimately leads to differential alterations in morphological parameters (Seneviratne *et al*., 2017) including changes in the leaf area, shoot length and root length (Pant *et al*., 2014), eventually caused by shifts in the physiological milieu attributed to plant growth performance (Doni *et al.,* 2014). These alterations also include numerous anatomical modifications in plants, for instance, dissolution and reduction of parenchymatous cells, mesophyll cells, and decrease in the number of xylem vessels as well as in the diameter of root stem and leaf (Batool *et al.*, 2015).

One of the most devastating metalloids, Arsenic (As) was reported to cause stress in rice seedlings by reducing the root length, and shoot length (Shri *et al.,* 2009; Upadhyay *et al*., 2016) thus decreasing the plant height and root growth (Abedin *et al*., 2002).

As suggested in one study, arsenic exposure to rice seedling causes significant reduction in the germination percentage, root-shoot elongation and also in plant biomass (Shri *et al.,* 2009).

Arsenic affects cells of rice crop by causing breakage to the root epidermal cells and aerenchymatous cortex (Choudhury *et al.,* 2011). Study conducted by Deng *et al*. (2010) demonstrated clearly how As caused structural changes in root anatomy by reducing the diameter of adventitious roots, and by forming the arenchyma and densely packed suberized epidermal cell wall that acted as a barrier.

Study on brown rice treated with As and Cd revealed that arsenic concentration had diminished from bottom to the top of brown rice whereas, Cd increased in the first node. Different nodes marked the significant difference between As and Cd in their accumulation and barrier capacities (Feng *et al.,* 2017). Verma *et al*. (2003) demonstrated that reduction in root length in rice seedlings occurs due to Cd toxicity. Cd also significantly suppressed the seed germination (Mahmood *et al.,* 2007; Eshagberi, 2012).

Li *et al*. (2014) working with four genotype of rice (maintainer lines Yixiang B and E2B, restorer lines R892 and Mianhui725), observed that new roots had developed in the cortex region in all genotypes and the diameter and frequency of roots differed significantly under low Cd concentrations. However rice seeds, treated with 100 μM Cd, generated oxidative stress, significantly slowed down the root-shoot elongation and also repressed the root-shoot fresh weight (Liu *et al*., 2013).

Moreover, another toxic element, Hg, was found to cause a significant reduction in root-shoot biomass in a hydroponic set up with rice plants (Wang *et al.,* 2014), as roots have the strongest potential for enrichment with HMs (Singh *et al*., 2013; Jin *et al*., 2015). Another hazardous metal Pb when accumulates in rice crop, decreases the dry biomass (Zeng *et al.,* 2007).

The toxic metalloid Arsenic exposures: 100 mM As (III) and 500 mM As (V) - to the rice plant were demonstrated to enhance MDA content in root and shoot signifying oxidative stress, as lipid peroxidation (Shri *et al*., 2009) consecutively altered the membrane permeability with an increased ion leakage (Mishra *et al*., 2011; Kumar *et al.,* 2014).

Cd, reportedly, causes enzymatic and metabolic modification in plants (Parmar *et al.,* 2013). The activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and dehydroascorbate reductase (DHAR) increase with an increase in Cd exposure to the rice varieties (Iqbal *et al*., 2010). Moreover, Cd treatment to the rice seedlings induced the ROS accumulation by increasing the level of malondialdehyde (MDA) and H_2O_2 (hydrogen peroxide) thus inhibited the antioxidant capacity in both roots and shoots.

Huang *et al*. (2008) reported that an increase in ROS production with supplementation of Pb ions occurs and that affects root cell viability, accelerating the cell death in rice seedling.

2.2 Effect of lead stress on crops

2.2.1 Germination and early growth of seedlings

Lead adversely affects seed germination higher concentrations of Pb^{2+} (Zeng *et al*., 2006). Hosseini *et al*. (2007) conducted an experiment and found that lead caused a decrease on germination in two different rice cultivars (Pf 7045 91 and Hyola 401). By increasing lead concentration more increasing in germination on Pf than Hyola has been shown. Growth parameters on both cultivars have been decreased by lead effect. The result show that germination on Pf was more resistant than the Hyola and at vegetative stage in Hyola has more resistant than the Pf.

Awan *et al*. (2015) seeds of the rice cultivars KSK-133, NIAB-IR-9, Basmati-385 (B-385) and Shaheen Basmati (SB) were treated with different concentrations of lead chloride ($PbCl_2$) (0, 250, 500, 1000 and 2000 ppm) to evaluate the effect on germination and seedling growth. Lead had no effect on germination percentage, except cv. SB at 2000 ppm. Germination rate decreased with increasing lead concentrations.

Jasmin *et al*. (2019) conducted a pot experiment to evaluate the effect of lead (50, 100, 150 and 200 mg kg^{-1}) on rice (*Oryza sativa*) and remediation of metal contamination by applying cow dung, poultry litter and lime to alleviate lead toxicity. The lengths, fresh and dry weights of shoot, root and macronutrients decreased with increasing level of lead compared to the control. The maximum reduction was observed in the pots treated with 200 mg kg^{-1} lead (19.50 and 20.03% for grain, 17.15 and 19.75% for shoot and 17.96 and 30.02% for root on the fresh and dry weight, respectively). On the other hand lead concentration in roots and shoots were increased with increasing lead treatment compared to the control. Treatments of the amendments (cow dung, poultry litter and lime) had positive effects though cow dung outshining the rest of them. This particular organic matter had considerable decreasing impacts in lead uptake by rice. Cow dung treated pots increased fresh and dry weight by 31.48 and 32.07% for grain, 14.08 and 35.30% for

shoot and 57.09 and 34.48% for root compared to pot treated with 100 mg kg^{-1} lead. Cow dung remediated lead concentration by 48.85, 65.00 and 62.00% for grain, shoot and root, respectively.

2.2.2 Effect of lead on morpho-physiological parameters

Yang *et al*. (2000) found a rapid development of adventitious roots and 10-fold higher root biomass with low Pb and higher oxalate contents in tolerant rice cultivars than sensitive ones which suggest that oxalate compounds are involved in reduced uptake of Pb. They further accentuated that exogenous feeding of oxalate to the growing medium ameliorate the Pb-induced root inhibition in rice.

Verma and Dubey (2003) conducted an experiment at Varanasi that seedlings of two rice (*Oryza sativa* L.) cultivars were raised in sand cultures under 500 and 1000 mM $Pb(NO_3)_2$ in the medium, lengths as well as weights of roots and shoots decreased with increase in Pb concentration.

About 1216, 179, and 62 times higher lead contents were recorded in roots, stems and leaves, respectively, than grains at maturity (Liu *et al*., 2003). So, a great variation exists among lead concentration within plant parts. Normally, lead concentration is recorded from maximum to minimum in the following order: root > shoot > ear (up to heading stage) > grain (at ripening stage).

Lead adversely affects root/shoot ratio, and their fresh and dry weight in rice were more at higher concentrations of Pb^{2+} (Zeng *et al*., 2006).

Conversely, Li *et al*. (2007) found significant reductions in rice growth and yield at 1200 mg of lead kg^{-1} of soil than control and found lower Pb^{2+} contents in rice grains than root, shoot and leaves.

A significant inhibition in morphological response and photosynthetic pigments with higher bioaccumulation of Pb in roots and ultrastructural aberrations in pollens were observed in two contrastive rice cultivars Pokkali and IRRI-112 against Pb stress (Arce and Yllano, 2008).

Li *et al*. (2012) exposed to four levels of lead (Pb) stress (0, 50, 100 and 200 µM) to assess effects on plant growth. Under Pb stress conditions, endophyte-infected

seedlings had greater shoot length but lower root length compared to non-infected controls, and endophyte-infected seedlings had greater dry weight in the 50 and 100 µM Pb treatments.

Khan *et al*. (2021) from Korea revealed that soil was contaminated with a solution containing 0.6 mM or 1.2 mM Pb four weeks prior to transplanting. Then, 4-week-old rice seedlings of Tunnae, Ilmi, Yasmen, Mashkab and Amber Barka were transplanted into the contaminated soil and grown until maturity. The results showed that a high concentration of lead (1.2 mM) induced significant reduction in the plant height, number of tillers, number of panicles plant^{-1} and the number of spikelets panicle^{-1} in Pb-sensitive rice cultivars, while in Pb-tolerant cultivars, a balanced growth of plants and non-significant change in the major yield components were recorded. However, all rice cultivars showed a reduced biomass dry weight. Interestingly, the rice cultivars Tunnae and Mashkab exhibited a high degree of tolerance towards Pb stress, with a balanced plant height, number of tillers, number of panicles and number of spikelets plant^{-1}.

2.2.3 Effect of lead on biochemical parameters

Yang *et al*. (2000) found that Pb inhibits upregulates ROS production; however, enhanced Ca^{2+} accumulation, myelin basic protein (MBP) kinase (a signaling pathway for systematic responses under stress conditions) activities, calcium-dependent protein kinase (CDPK) (involved in rapid biochemical activation under abiotic stresses) inhibits Pb-induced cell death and mitogen-activated protein (MAP) kinase activation (one of the main pathways by which extracellular stimuli are transduced into intracellular responses) (Huang and Huang, 2008).

Verma and Dubey (2003) conducted an experiment at Varanasi at found that Pb-treated seedlings showed elevated levels of lipid peroxides with a concomitant increase in the activities of the enzymes superoxide dismutase (SOD), guaiacol peroxidase, ascorbate peroxidase and glutathione reductase compared to controls. Though Pb was readily absorbed by growing seedlings, its localization was greater in roots than shoots. The level of Pb accumulation in seedlings was far higher than the supplied one. Seedlings grown for 5-20 days in presence of 1000 mM $Pb(NO_3)_2$ showed about 21-177% increase in the level of thiobarbituric acid reacting substances (TBARS) in shoots indicating enhanced

lipid peroxidation compared to controls. With increase in the level of Pb treatment in situ peroxidases showed more increase in activity than SOD. Under both controls as well as Pb treatments roots maintained higher activity of these enzymes than shoots. About 87-100% increase in SOD activity, 1.2-5.6 times increase in guaiacol peroxidase activity and 1.2-1.9 times increase in ascorbate peroxidase activity was observed in the roots of seedlings grown for 15 days in presence of 1000 mM Pb in the medium. Under similar treatment conditions about 128-196% increase in glutathione reductase activity was recorded in roots and 69-196% increase in shoots compared to control grown seedlings. Pb treatment resulted in a decline in catalase activity in roots whereas in shoots catalase activity increased in seedlings grown at moderately toxic Pb (500 mM) level whereas a highly toxic Pb (1000 mM) level led to a marked inhibition in enzyme activity. Two catalase isoforms were etected in roots and three in shoots of the seedlings.

The effect of Pb toxicity on photosynthetic machinery varies with rice genotype. Normally, chlorophyll b is more prone to distortion under Pb-stressed conditions than chlorophyll a (Xiong *et al*., 2006).

The process of chlorophyll cessation is catalyze by chlorophyllase, pheophorbide oxygenase, red chlorophyll catabolite reductase, and Mg-dechelatase that dissociates it in to magnesium, phytol, and product of porphyrin after primary cleavage, that is also responsible for chlorophyll bleaching (loss of green color) (Harpaz-Saad *et al*., 2007).

Lead toxicity inhibited photosynthesis adversely by distortion of chloroplast structure, reduction in rate of chlorophyll, plastoquinone and carotenoid synthesis, disruption of electron transport chain, cause CO_2 deficiency by stomatal closure, and reduction in the enzymatic activity of Calvin cycle (Xiong *et al*., 2006; Singh *et al*., 2010).

The main reasons of reduced photosynthetic activity due to Pb may be the affinity of lead for protein N and S ligands; cause damage to chloroplastic ultrastructure (Islam *et al.,* 2007); hindrance in the electron transport chain (ETC) reactions (Qufei and Fashui, 2009); breakdown of chlorophyll contents by enhanced activity of chlorophyllase (Liu *et al.,* 2008); inhibition and substitution of Mg and Fe by Pb (divalent cations) in chlorophyll (Chatterjee *et al.,* 2004; Cenkci *et al.,* 2010); reduced activities of ferredoxin

$NADP^+$ reductase as well as delta-aminolevulinic acid dehydratase at the source of chlorophyll production (Gupta *et al*., 2009); reduced CO_2 levels due to stomatal closure (Romanowska *et al.,* 2006); plastoquinone and carotenoid inhibition (Chen *et al.,* 2007; Cenkci *et al.,* 2010); and inhibited enzymatic catalysis of Calvin cycle (Liu *et al.,* 2008).

Ashraf *et al*. (2017) study assessed the biochemical responses, in three different fragrant rice cultivars i.e., Meixiangzhan-2, Xinagyaxiangzhan and Basmati-385. Plants were exposed to 400, 800, and 1,200 ppm of Pb while pots without Pb were taken as control (0 ppm). All rice cultivars readily taken up the Pb contents from soil to roots and transported upward in different proportions with maximum in roots followed by stemss, leaves, ears and grains. Higher proportions of Pb contents in above ground plant parts in Xinagyaxiangzhan possibly lead to maximum losses in this cultivar than other two cultivars.

2.2.4 Effect of lead on antioxidant enzymes

Verma and Dubey (2003) conducted an experiment at Varanasi at found that highly toxic Pb (1000 mM) level led to a marked inhibition in enzyme activity Results suggest that Pb induces oxidative stress in growing rice plants and that SOD, peroxidases and GR could serve as important components of antioxidative defense mechanism against Pb induced oxidative injury in rice.

Hosseini *et al*. (2007) conducted an experiment and found that enzymatic activity of catalase and peroxidase in root and shoot on both rice cultivars (Pf 7045 91 and Hyola 401) by increasing lead concentration has been increased.

Li *et al*. (2012) exposed to four levels of lead (Pb) stress (0, 50, 100 and 200 µM) to assess effects on antioxidant enzyme activity. Antioxidant activity was either higher or unchanged in the infected seedlings due to responses to the different Pb concentrations. These results suggest that the endophytic fungus improved rice growth under moderate Pb levels by enhancing photosynthesis and antioxidant activity relative to non-infected rice.

Ashraf *et al*. (2017) study assessed the biochemical responses, in three different fragrant rice cultivars i.e., Meixiangzhan-2, Xinagyaxiangzhan and Basmati-385. Plants

were exposed to 400, 800, and 1,200 ppm of Pb while pots without Pb were taken as control (0 ppm). Higher proportions of Pb contents in above ground plant parts in Xinagyaxiangzhan possibly lead to maximum losses in this cultivar than other two cultivars; while less damage in Basmati-385 might be related to strong anti-oxidative defense system and lower proportions of Pb contents in its aerial parts.

Khan *et al*. (2021) from Korea revealed that soil was contaminated with a solution containing 0.6 mM or 1.2 mM Pb four weeks prior to transplanting. Differential enzymatic antioxidant activity, with catalase (CAT) and peroxidase (POD) being the most active. In addition, the proline accumulation and sucrose content increased concomitant with an increase in the Pb concentration, while the total protein and chlorophyll contents significantly decreased. Of all the soluble sugars analyzed, sucrose was the most abundant in response to Pb treatment. Therefore, all results collectively suggest that the tolerance to Pb-induced oxidative stress observed in Tunnae and Mashkab could be a result of a synergetic action of both enzymatic and non-enzymatic antioxidant systems, leading to a balanced reduction–oxidation status in rice.

CHAPTER-III

MATERIAL AND METHODS

A pot experiment was conducted in the laboratory of Department of Plant Physiology at Institute of Agricultural Sciences, Banaras Hindu University with five rice cultivars and three lead nitrate levels with three replications during *kharif* 2020. The materials used and methods applied are discoursed briefly in this chapter.

3.1 Experimental methods

Table 3.1: Experimental details

Experimental design	CRD
Number of cultivars	Five
Lead nitrate levels	Three
Number of replications	Three
Total number of pots	Fourty five

Treatment details

Table 3.2: Treatment details

Treatment	**Treatment detail**
Rice Cultivars (V)	
V_1	HUR 1304
V_2	HUR 36
V_3	HUR 3022
V_4	HUR 1309
V_5	HUR 109
Lead nitrate	
L_0	Control
L_1	2 mM
L_2	4 mM

3.2 Experimental material

3.2.1 Seed source

Pure, healthy and disease-free seeds of rice (*Oryza sativa* L.) cultivars HUR 1304, HUR 36, HUR 3022, HUR 1309 and HUR 109 were obtained from Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

3.2.2 Lead nitrate source

Lead nitrate [Pb $(NO_3)_2$] was obtained from Department of Plant Physiology, Institute of Agricultural Sciences, BHU, Varanasi.

3.2.2.1 Preparation of lead nitrate solution and application

Lead nitrate [Pb $(NO_3)_2$] solution of 2mM and 4 mM was prepared as per standard procedure and after that is was used for seed treatment as per treatment.

3.2.3 Sterilization of pots

Forty five number of pots were obtained from Department of Plant Physiology, Institute of Agricultural Sciences, BHU, Varanasi. The pots were washed using lab detergent and rinsed initially with tap water then finally with distilled water. The washed pots were placed in oven at sun light for 48 hours for dry sterilization.

3.6 Sampling procedure and observations recorded

Morphological, physiological and biochemical parameters were recorded under control, lead nitrate under different cultivars. Each pot contains 20 seedlings and these seedlings were utilized for determination of morphological and growth parameters shoot and root. For biochemical analysis seedlings were removed carefully from pots with intact roots and shoots without any damage.

3.7 Morphological parameters

The morphological parameters of roots, shoots and leaves were analysed separately. The morphological data were taken at 15 and 30 DAS.

3.7.1 Germination percentage

Germination percentage was calculated at 5 DAS using the following formula:

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds in the pot}}{\text{Total number of seeds sown in pot}} \times 100$$

3.7.2 Radicle length (cm)

Radicle length was obtained by taking average of radicle length of three plants obtained from the three replications under each treatment. Radicle length is expressed in centimetres. The length of radicle was measured using a thread and measuring from the base of the radicle to the plumule and expressed in centimetres.

3.7.3 Plumule length (cm)

Plumule length was obtained by taking average of plumule length of three plants obtained from the three replications under each treatment. Plumule length is expressed in centimetres. The length of plumule was measured using a thread and measuring from the base of the plumule to the main growing tip and expressed in centimetres.

3.7.4 Dry matter seedling^{-1} (g)

Dry weight of one plant from each replication under each treatment was measured and averaged to determine the dry matter of seedling. Seedling samples were blot dried to remove any moisture present on the surface then were placed inside an envelope for drying inside an oven. The plants are dried in a hot air oven at 100°C for an hour to kill the metabolic activities followed by constant temperature of 70°C for a period of 72 hours. Weighing was made using an electronic weighing balance regularly till a constant weight was achieved.

3.7.5 Number of leaves seedling^{-1}

Number of leaves seedling^{-1} was counted at 30 days after sowing.

3.8 Biochemical properties

The biochemical parameters were measured separately for leaves/plumule and radicle at 15 days after sowing.

3.8.1 Chlorophyll content (µg g^{-1} FW)

3.8.1.1 Reagents used in Chlorophyll estimation

Dimethyl Sulfoxide (DMSO)

3.8.1.2 Procedure

The chlorophyll content of leaf sample was determined by method given by Hiscox and Israelstam (1979). 50 mg of leaf sample was weighed and made into small pieces. These pieces are poured in a test tube containing 5 mL DMSO (dimethyl sulfoxide) and kept it in water bath at 70°C for 2 hours along with a control test tube containing only DMSO (dimethyl sulfoxide) with-out any leaf sample. After 2 hours the test tubes were removed out of the water bath, cooled and volume was made up to 10 mL using DMSO (dimethyl sulfoxide) solution. Absorbance reading were recorded at 663 nm and 645 nm with the help of spectrophotometer (SCC-177 scanning mini spectrophotometer). The chlorophyll content was calculated using the following formulae:

$$\text{Chlorophyll 'a' content (µg g}^{-1}\text{ FW)} = \frac{(12.7 \times A663 - 2.69 \times A645) \times \text{Volume of sample}}{1000 \times \text{Weight of sample}}$$

$$\text{Chlorophyll 'b' content (µg g}^{-1}\text{ FW)} = \frac{(22.9 \times A645 - 4.68 \times A663) \times \text{Volume of sample}}{1000 \times \text{Weight of sample}}$$

$$\text{Total Chlorophyll content (µg g}^{-1}\text{ FW)} = \frac{(20.2 \times A645 + 8.02 \times A663) \times \text{Volume of sample}}{1000 \times \text{Weight of sample}}$$

where, A_{645}, A_{663} are the absorbance readings at 645 and 663 nm wavelengths respectively.

3.8.2 Carotenoids (µg g^{-1} FW)

Carotenoids in plant samples were analyzed as per standard procedure.

3.8.3 Membrane stability index (%)

Membrane stability index (MSI) was determined by the method described by Sairam (1994). For the measurement of electrolyte leakage, leaves from plant grown at different lead nitrate concentrations were excised. Five grams fresh weight of leaves were cut into small pieces (about 2 cm) and washed with distilled water to remove electrolyte from injured cell at the cut edge and any surface adhering electrolyte. After wiping out of extra water from leaf surface with filter paper they were placed in test tube containing 20 mL of distilled water in two sets. Samples for each genotype were replicated thrice. One set of test tubes were placed in a water bath at 40ºC for one h and another kept at 100ºC in boiling water bath for 15 minutes and their respective electrical conductivities C_1 and C_2 were measured using conductivity meter (Systronics conductivity meter, 306). Membrane thermostability was measured using the following formulae:

Membrane Stability Index = $[1-(C_1/C_2)] \times 100$

3.8.4 Total soluble sugar (mg g^{-1} FW)

Sucrose Standards for Colorimetric Detection

Dilute 10 µL of the 100 nmole/µL Sucrose Standard Solution with 990 µL of Glucose Assay Buffer to prepare a 1 nmole/µL standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 nmole/µL Sucrose standard solution into a 96 well plate, generating 0 (assay blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Glucose Assay Buffer to each well to bring the volume to 50 µL.

Sucrose Standards for Fluorometric Detection

Prepare a 1 nmole/µL standard solution as for the Colorimetric Assay. Dilute 20 µL of the 1 nmole/µL standard solution with 180 µL of Glucose Assay Buffer to generate a 0.1 nmole/µL standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 0.1 nmole/µL Sucrose standard solution into a 96 well plate generating, 0 (assay blank), 0.2, 0.4, 0.6,

0.8, and 1.0 nmole/well standards. Add Glucose Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation

Liquid samples can be measured directly. Bring samples to a final volume of 50 µL with Glucose Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

For sucrose detection, set up duplicate wells for each sample in which glucose is measured. These wells will be used to determine total glucose.

Assay Reaction

1. Add 2 µL of Invertase to each of the sucrose samples and to the sucrose standards. Add 2 µL of Glucose Assay Buffer to the glucose sample. Incubate the plate at 37 °C for 30 minutes.
2. Set up the Master Reaction Mix according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).

3.8.5 Soluble protein (µg g^{-1})

100 mg fresh weight was taken and homogenized in 5.0 mL 0.1 M phosphate buffer having pH 7.5, using chilled pestle and mortar. The extract was centrifuged at 10,000 × g for 20 minutes. Extraction was repeated twice with 2 mL extraction medium. All the supernatants were pooled and final volume was made to 10 mL.

Quantification (Bradford method)

Protein in the crude extract was determined according to the Coomasie Brilliant Blue G-250 dye binding method (Bradford, 1976).

Bradford regeant Coomasie Brilliant Blue G-250 was dissolved in 50 ml ethanol, and to it 100 mL o-phosphoric acid (85% w/v) was added. The total volume was made up to 1 Litre (L) with double distilled water. The solution was filtered through Whatman No. 1 filter paper and stored in dark at room temperature.

Procedure

200 µL of extracted protein was taken. 3.0 mL Bradford dye was added and the samples were incubated at 37˚C for 5 minutes in dark. The absorbance was observed at 595 nm. Protein concentration was calculated with the help of standard curve.

Preparation of standard curve

Standard was prepared by taking quantities (10 to 100 µg) of bovine serum albumin (BSA) in a total of 1 mL and treating them in the same way as the test solution. Amount of protein was expressed as mg g^{-1} fresh weight of the leaf material.

3.8.6 Alpha amylase (mg maltose g^{-1} h^{-1} FW g^{-1})

For the estimation of the α-amylase (EC 3.2.1.1) activity in the weighed amount (100 mg) of endosperm of germinating rice seeds, method of Bernfeld (1955) was followed. For extraction of amylase the method described by Goldstein and Jennings (1975) was used with slight modifications. The unit activity is defined as the micro g maltose liberated in 3 minutes at 30°C by I mL enzyme extract.

Following chemicals and reagents were used to measure Amylase activity

a) Tris-HCL (0.05M, pH 7.6): 0.05 M solution of Tris HCL was prepared by dissolving (7.880g of Tris HCL in 100 mL distilled water).

b) 2-mercaptoethanol (5 mM): 5 mM solution of 2-mercaptoethanol in Tris-HCL buffer (0.05 M) was prepared by adding 0.0342 mL of 2-mercaptoethanolin to it and finally adjusting volume to100 mL.

c) 3, 5- dinitrosalicyclic acid (DNS; 1%): 1g 3, 5- dinitrosalicyclic acid (DNS) was dissolve in the 20mL of NaOH (2N). 50mL of distilled water was added to it, after this 30 g of Rochelle salt (potassium sodium tartrate) was added and final volume was made up to 100 mL. This solution was protected from carbon dioxide gas by keeping it in to dark and cool place (2N NaOH: 8 g of NaOH in 100 mL of distilled water).

d) Starch solution (1%) in phosphate buffer (0.2 M, pH 6.9): 1g of starch was dissolve in 100 mL of phosphate buffer to get 1% starch solution.

e) $CaCl_2$ (6M): 6M solution of $CaCl_2$ was prepared by dissolving (88.2 g of $CaCl_2$ in 100 mL distilled water).

Procedure:

One hundred mg blotted-dry endosperms were taken out from germinated seeds of rice. Sample were homogenized for 60 sec in 10 mL precooled 0.05 M Tris-HCL buffer, pH 7.6, containing 5 mM 2-mercaptoethanol in a precooled mortar and pestle. The homogenates were centrifuged at 10,000 g at 4°C for 10 minutes. Supernatant represented the enzyme (amylase) extract. The supernatant was collected and the volume was maintained up to 10 mL by adding Tris-HCL buffer. Two aliquots of the supernatant were removed and one of them (For α-amylase: supernatant enzyme 0.5 ml, H_2O, 0.0 mL and $CaCl_2$ 0.5 mL) was heated for 5 minutes at 70°C in water bath. Presence of 3 mM $CaCl_2$ inactivates β-amylase. Solution was then cooled to 0°C (Bilderback, 1973) and another aliquot (total amylase enzyme 0.5 mL, H_2O 0.5 mL and CaCL2 0.0 mL) was incubated for 5 minutes at 25°C. After this 1 mL (1%) of starch solution was taken and added into the incubated solution. The same mixture was kept for incubation. After incubation at 20°C for 5 minutes 3 mL DNS solution was added for the suppression of enzyme activity. Test tubes containing reaction mixtures were kept in boiling water bath for 5 minutes then cooled with the help of running tap water and final volume of solution in test tubes was raised up to 10 mL by adding distilled water. In control, instead of 1 mL enzyme extract distilled water was used. Absorbance was read at 540 nm. Standard curve of maltose was used for calculating the activity of amylases.

3.8.7 Catalase (EU mg^{-1} Protein min^{-1})

The activity of enzyme catalase was measured in leaf samples at 15 days after sowing. Enzyme was assayed according to the protocol given by Aebi *et al*. (1983).

Reagents:

1. 0.1 M Phosphate buffer, pH 6.4
2. 1 % (v/v) H_2O_2

Procedure:

One hundred mg leaf samples were taken and homogenized in 5 mL of 0.1 M phosphate buffer in a chilled pestle and mortar. The crude extract was centrifuged at 10,000 g for 20 minutes at 4 °C. The enzyme extract was stored at low temperature until completion of enzyme assay. The activity of enzyme was assayed by taking 2.6 mL, 0.1 M phosphate buffer, 0.1 mL enzyme extract and 0.1 mL 1% H_2O_2. The reaction mixture was mixed rapidly at room temperature. A blank was prepared similarly in which 0.1 M phosphate buffer was added in reaction mixture instead of enzyme extract. Changes in absorbance at 240 nm (δ A 240) at an interval of 15 seconds for 2 minutes were noted. The enzyme activity per g FW and was estimated using extinction coefficient 43.6 for H_2O_2 decomposition. It was also estimated on per mg protein basis and was expressed according to the formula:

EU mg^{-1} protein = δ A 240/min × 1000 / 43.6 × mg protein mL^{-1} reaction mixture. The EU was expressed on per g fresh weight basis as well as on the basis of per mg protein (specific activity).

3.8.8 Super oxide dismutase (µg g^{-1} x 10^2 g^{-1} FW min^{-1})

The activity of superoxide dismutase enzyme was measured in leaf samples at 15 days after sowing according to the protocol given by Dhindsa *et al*. (1981).

Reagents:

Potassium phosphate buffer (0.1 M, pH 7.5)

Solution of potassium dihydrogen phosphate 0.1 M (Solution A) and dipotassium hydrogen phosphate 0.1 M (Solution B) were prepared by dissolving 13.6 g L^{-1} and 17.4 L^{-1} salts, respectively. 16 mL of solution A and 84 mL of solution B were mixed and the pH of the mixed solution was adjusted to pH 7.5

L- methionine (200 mM)

L- methionine (0.298 g) was dissolved in distilled water and the volume was made to 10 mL.

Nitro blue tetrazolium (NBT) (2.25 mM)

NBT (0.0184) was dissolved in distilled water and the volume was made to 20 mL and kept in air tight vial.

EDTA (3 mM)

EDTA (0.0560 g) was dissolved in distilled water and the volume was made to 50 mL.

Riboflavin (60 µM)

Riboflavin (0.0023 g) was dissolved in distilled water and the volume was made to 100 mL with distilled water and stored in amber colour bottle at 4 °C in a refrigerator.

Sodium carbonate (1.5 M)

Sodium carbonate (15.9 g) was dissolved in distilled water and the volume was made to 100 mL.

Procedure:

Plant sample (100 mg) was homogenized with 5 mL extraction buffer (0.1 M phosphate buffer pH 7.5 containing 0.5 mM EDTA). The homogenate was centrifuged in a cooling centrifuge machine (REMI, C-24) at 10,000 g for 10 minutes. After centrifugation, supernatant was collected and this supernatant was used as enzyme source. Three mL of the reaction mixture containing 0.1 M of 1.5 M sodium carbonate, 0.2 mL of 200 mM methionine, 0.1 mL of 2.25 mM NBT, 01 mL of 3 mM EDTA, 1.5 mL of 100 mM potassium phosphate buffer, 1 mL of distilled water and 0.1 mL of enzyme extract were taken in test tubes in 3 replications for each enzyme sample. Two tubes without enzyme extract were taken as control. Three set of test tubes were prepared. The reaction was started by adding 0.1 mL riboflavin (60 µM) in all the sets of test tubes and placing the 2 sets of tubes (one in which enzyme was added and the other in which enzyme was not added) below a light source of two fluorescent lamps for 15 minutes. Reaction was stopped by switching off the light and covering the sets of tubes by a black cloth. The set of tubes without enzyme extract developed maximum color. A non-

irradiated set in which light source was not supplied but contained enzyme extract was kept in dark and it did not develop colour and served as blank. Absorbance of all the sets of test tube was recorded at 560 nm using a spectrophotometer (ELICO, SL 196). Enzyme unit (EU) was calculated as per the formula given below:

EU = absorbance without enzyme in light- (absorbance with enzyme in light absorbance in dark)/ absorbance without enzyme in light /2

The EU was expressed on per g fresh weight basis as well as on the basis of per mg protein (specific activity).

3.8.9 Ascorbate peroxidase (EU mg^{-1} Protein min^{-1})

Ascorbate peroxidase activity was assayed in leaf samples at 15 days after sowing. Ascorbate peroxidase was assayed as per the protocol of Nakano and Asada (1981).

Reagents

1. Ascorbic acid (3.0 mM)
2. EDTA (3.0 mM)
3. Hydrogen peroxide (3.0 mM)
4. Phosphate buffer (100 mM, pH 7.0)

Solution A: Potassium dihydrogen phosphate (6.80 g) was dissolved in water and the volume was made up to 500 mL with double distilled water.

Solution B: Di-potassium hydrogen phosphate (8.71 g) was dissolved in water and the volume was made up to 500 mL with double distilled water.

Phosphate buffer (100 mM, pH 7.0) was prepared by mixing 39 mL of solution A and 61 mL of solution B and final pH was adjusted with the help of pH meter.

Procedure

Enzyme extract for APX was prepared by grinding 100 mg sample with 1.0 mL of extraction buffer (0.1 M phosphate buffer, pH 7.5 containing 0.5 mM EDTA and 1 mM ascorbic acid). The homogenate was centrifuged in centrifuge at 10000 x g for 10

minutes at 4ºC. After centrifugation, supernatant was collected and this supernatant was used as enzyme source.

The 3 mL reaction mixture contained:

- ✓ 50 mM potassium phosphate buffer (pH 7.0) (1.5 mL of 100 mM)
- ✓ 0.5 mM ascorbic acid (0.5 mL of 3.0 mM)
- ✓ 0.1 mM EDTA (0.1 mL of 3.0 mM)
- ✓ 0.1 mM H_2O_2 (0.1 mL of 3.0 mM)
- ✓ 0.2 mL enzyme
- ✓ 0.6 ml water (to make a final volume of 3.0 ml)

The reaction was started with the addition of 0.1 ml of hydrogen peroxide. Decrease in absorbance for a period of 30 second was measured at 290 nm in an UV-visible spectrophotometer.

Enzyme activity was calculated in terms of amount of ascorbic acid oxidized (initial absorbance – final absorbance = quantity of ascorbic acid oxidized) per minute per mg protein.

3.9 Statistical analysis

The experiments were laid out in CRD (completely randomized design) with three replications per treatment. In this study, the data obtained from the experiment under different treatments with respect to various parameters were subjected to analysis of variance (ANOVA) with treatments as sources of variation. Values of different parameters were expressed as the mean ± standard error. A difference was considered statistically significant when the p-value was less than 0.05 ($p \leq 0.05$). All analysis were performed with MS Excel-2016 (windows 11) and online statistical software WASP - Web Agri Stat Package 2.0 (ICAR-CCARI, Goa).

CHAPTER-IV

EXPERIMENTAL FINDINGS

The present pot experiment entitled **"Effect of Lead Levels on Germination attributes and Biochemical Parameters in Rice (*Oryza sativa* L.)"** during *kharif* season of 2019 at Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh. The five genotypes of rice HUR 1304, HUR 36, HUR 3022, HUR 1309 and HUR 109 were treated with two levels (2 mM and 4 mM) of lead nitrate and sown in pots to find out the effect of lead levels on these genotypes and in view to compare the damaging effect of lead. The results of investigation have been presented and interpreted in this chapter. The experimental results are presented under following broad headings:

4.1 Morpho-physiological parameters

4.2 Biochemical parameters

4.1 Morpho-physiological parameters

The Morpho-physiological parameters of rice crop were measured in terms of germination (%), radicle length (cm), plumule length (cm), dry matter seedling^{-1} (g) and number of leaves seedling^{-1}.

4.1.1 Germination (%)

Per cent germination showed non-significant differences among five genotypes under the influence of lead nitrate levels as presented in Table 4.1. Among treatment percent germination was found maximum in control, whereas the least per cent germination was observed in treatment L_2 (Pb $(NO_3)_2$ @ 4 mM). Among genotypes, non-significant differences in per cent germination was recorded and HUR 36 showed non-significantly higher percent germination (93.96%) than other genotypes in control condition. In all genotypes, L_2 showed the significant reduction in percent germination as compared to control. The maximum reduction in per cent germination was recorded in HUR 36 with 67.79% percent.

Interaction effect of different cultivars and lead nitrate levels found to be non-significant.

Table 4.1: The effect of lead levels on percent germination in rice (*Oryza sativa* (L.)) genotypes

Treatments	**V_1**	**V_2**	**V_3**	**V_4**	**V_5**
L_1	91.84	98.96	98.41	89.93	89.59
L_2	82.14	74.50	79.63	79.29	78.95
L_3	76.27	67.79	67.96	73.31	72.97

Treatments	**Germination (%)**
Rice cultivars	
V_1: HUR 1304	83.42
V_2: HUR 36	80.42
V_3: HUR 3022	82.00
V_4: HUR 1309	80.84
V_5: HUR 109	80.50
SEm±	4.75
LSD (*p*=0.05)	NS
Lead nitrate	
L_0: Control	93.75
L_1: 2 mM	78.90
L_2: 4 mM	71.66
SEm±	3.68
LSD (*p*=0.05)	7.52
Interaction (V x L)	NS

4.1.2 Radicle length (cm)

Lead stress caused a non-significant decrease in radicle length of every genotype at 15 DAS and DAS. Among treatments, radicle length was found longest in control, whereas the shortest radicle length was recorded in treatment L_2 (Pb $(NO_3)_2$ @ 4 mM) at both stages. Among genotypes, non-significant differences in radicle length were observed. HUR 1304 showed higher radicle length than other genotypes in control treatment at both the stages. Radicle length increased from 15 DAS to 30 DAS. The maximum reduction in radicle length was observed in genotype HUR 109 with 24.64 % and 6.90% at 15 DAS and 30 DAS respectively. The observation was depicted in Table 4.2.

Interaction effect of different cultivars and lead nitrate levels found to be non-significant.

Table 4.2: The effect of lead levels on radicle length (cm) in rice (*Oryza sativa* (L.)) genotypes

Radicle length (cm) at 15 DAS					
Treatments	**V_1**	**V_2**	**V_3**	**V_4**	**V_5**
L_1	20.08	19.66	20.63	18.02	15.96
L_2	18.29	14.08	16.61	16.23	14.18
L_3	18.61	12.36	14.02	15.70	12.79
Radicle length (cm) at 30 DAS					
Treatments	**V_1**	**V_2**	**V_3**	**V_4**	**V_5**
L_1	44.67	50.29	51.26	48.64	46.58
L_2	33.21	33.47	36.01	35.63	33.57
L_3	27.02	26.25	27.42	28.61	26.55

Treatments	**Radicle length (cm)**	
	15 DAS	**30 DAS**
Rice cultivars		
V_1: HUR 1304	6.33	11.65
V_2: HUR 36	5.12	12.22
V_3: HUR 3022	5.70	12.74
V_4: HUR 1309	5.55	12.54
V_5: HUR 109	4.77	11.86
SEm±	0.56	0.42
LSD (p=0.05)	NS	NS
Lead nitrate		
L_0: Control	6.29	16.10
L_1: 2 mM	5.29	11.46
L_2: 4 mM	4.90	9.06
SEm±	0.43	0.32
LSD (p=0.05)	0.88	0.66
Interaction (V x L)	NS	NS

4.1.3 Plumule length (cm)

Lead stress caused a non-significant decrease in plumule length of every genotype at 15 DAS and DAS. Among treatments, plumule length was found longest in control, whereas the plumule length was recorded in treatment L_2 (Pb $(NO_3)_2$ @ 4 mM) at both stages. Among genotypes, non-significant differences in plumule length were observed. HUR 1304 showed higher plumule length than other genotypes in control treatment at both the stages. Plumule length increased from 15 DAS to 30 DAS. The maximum reduction in plumule length was observed in genotype HUR 109 with 18.87 % and 9.76% at 15 DAS and 30 DAS respectively. The observation was depicted in Table 4.3.

Interaction effect of different cultivars and lead nitrate levels found to be non-significant.

Table 4.3: The effect of lead levels on plumule length (cm) in rice (*Oryza sativa* (L.)) genotypes

Plumule length (cm) at 15 DAS					
Treatments	**V_1**	**V_2**	**V_3**	**V_4**	**V_5**
L_1	33.34	32.78	34.09	30.56	27.77
L_2	30.92	25.22	28.64	28.14	25.36
L_3	23.97	18.00	19.58	21.19	18.41
Plumule length (cm) at 30 DAS					
Treatments	**V_1**	**V_2**	**V_3**	**V_4**	**V_5**
L_1	88.60	87.91	89.52	85.18	81.75
L_2	66.99	59.98	64.19	63.57	60.14
L_3	55.33	47.98	49.93	51.91	48.48

Treatments	**Plumule length (cm)**	
	15 DAS	**30 DAS**
Rice cultivars		
V_1: HUR 1304	9.80	23.44
V_2: HUR 36	8.44	21.76
V_3: HUR 3022	9.15	22.63
V_4: HUR 1309	8.88	22.29
V_5: HUR 109	7.95	21.15
SEm±	0.64	0.71
LSD (p=0.05)	NS	NS
Lead nitrate		
L_0: Control	10.57	28.86
L_1: 2 mM	9.22	20.99
L_2: 4 mM	6.74	16.91
SEm±	0.49	0.55
LSD (p=0.05)	1.00	1.12
Interaction (V x L)	NS	NS

4.1.4 Dry matter seedling^{-1} (g)

Lead stress caused a non-significant decrease in dry matter seedling^{-1} (g) of every genotype at 15 DAS and DAS. Among treatments, higher dry matter seedling^{-1} was found in control, whereas the least dry matter seedling^{-1} was recorded in treatment L_2 (Pb $(NO_3)_2$ @ 4 mM) at both stages. Among genotypes, non-significant differences in dry matter seedling^{-1} were observed. HUR 1304 showed higher dry matter seedling^{-1} than other genotypes in control treatment at both the stages. Dry matter seedling^{-1} increased from 15 DAS to 30 DAS. The maximum reduction in dry matter seedling^{-1} was observed in genotype HUR 109 with 20.93 % and 8.33% at 15 DAS and 30 DAS respectively. The observation was depicted in Table 4.4.

Interaction effect of different cultivars and lead nitrate levels found to be non-significant.

Table 4.4: The effect of lead levels on dry matter seedling^{-1} (g) in rice (*Oryza sativa* (L.)) genotypes

Dry matter seedling^{-1} (g) at 15 DAS					
Treatments	**V_1**	**V_2**	**V_3**	**V_4**	**V_5**
L_1	4.27	4.20	4.38	3.89	3.50
L_2	3.94	3.14	3.62	3.55	3.16
L_3	3.41	2.43	2.69	2.95	2.50
Dry matter seedling^{-1} (g) at 30 DAS					
Treatments	**V_1**	**V_2**	**V_3**	**V_4**	**V_5**
L_1	8.19	8.29	8.65	8.13	7.70
L_2	6.16	5.61	6.16	6.02	5.62
L_3	5.06	4.45	4.75	4.89	4.50

Treatments	**Dry matter seedling^{-1} (g)**	
	15 DAS	**30 DAS**
Rice cultivars		
V_1: HUR 1304	1.29	2.16
V_2: HUR 36	1.09	2.04
V_3: HUR 3022	1.19	2.17
V_4: HUR 1309	1.15	2.12
V_5: HUR 109	1.02	1.98
SEm±	0.09	0.07
LSD (p=0.05)	NS	NS
Lead nitrate		
L_0: Control	1.35	2.73
L_1: 2 mM	1.16	1.97
L_2: 4 mM	0.93	1.58
SEm±	0.07	0.05
LSD (p=0.05)	0.14	0.11
Interaction (V x L)	NS	NS

4.1.5 Number of leaves seedling^{-1}

Number of leaves seedling^{-1} showed non-significant differences among five genotypes under the influence of lead nitrate levels as presented in Table 4.5. Among treatment number of leaves seedling^{-1} was found maximum in control, whereas the least number of leaves seedling^{-1} was observed in treatment L_2 (Pb $(NO_3)_2$ @ 4 mM). Among genotypes, non-significant differences in number of leaves seedling^{-1} was recorded and HUR 3022 showed higher number of leaves seedling^{-1} (3.85) than other genotypes in control condition. In all genotypes, L_2 showed the maximum reduction in number of leaves seedling^{-1} as compared to control. The maximum reduction in number of leaves seedling^{-1} was recorded in HUR 109 with 8.11 percent.

Interaction effect of different cultivars and lead nitrate levels found to be non-significant.

Table 4.5: The effect of lead levels on number of leaves seedling^{-1} (g) in rice (*Oryza sativa* (L.)) genotypes

Treatments	**V_1**	**V_2**	**V_3**	**V_4**	**V_5**
L_1	14.93	15.11	15.77	14.81	14.03
L_2	10.21	9.30	10.21	9.99	9.32
L_3	9.22	8.12	8.66	8.91	8.21

Treatments	**Number of leaves seedling^{-1}**
Rice cultivars	
V_1: HUR 1304	3.82
V_2: HUR 36	3.61
V_3: HUR 3022	3.85
V_4: HUR 1309	3.75
V_5: HUR 109	3.51
SEm±	0.17
LSD (*p*=0.05)	NS
Lead nitrate	
L_0: Control	4.98
L_1: 2 mM	3.27
L_2: 4 mM	2.87
SEm±	0.13
LSD (*p*=0.05)	0.27
Interaction (V x L)	NS

4.2 Biochemical properties

The biochemical properties of rice crop were measured in terms of chlorophyll content (µg g^{-1} FW), carotenoids (µg g^{-1} FW), membrane stability index (%), total soluble sugar (mg g^{-1} FW), alpha amylase (mg maltose g^{-1} h^{-1} FW), catalase (EU mg protein min^{-1}), super oxide dismutase (µg g^{-1} x 10^2 g^{-1} FW min^{-1}) and ascorbate peroxidase (EU mg^{-1} protein min^{-1}).

4.2.1 Chlorophyll content (µg g^{-1} FW)

Lead stress caused a significant effect on chlorophyll content of every genotype on chlorophyll A and B. Among treatments, higher chlorophyll content was found in control, whereas the least chlorophyll content was recorded in treatment L_2 (Pb $(NO_3)_2$ @ 4 mM) on chlorophyll A, B and total chlorophyll. Among genotypes, significant differences in chlorophyll content were observed. HUR 1304 showed significantly higher chlorophyll content than other genotypes in control treatment on chlorophyll A, B and total chlorophyll. The maximum reduction in chlorophyll content was observed in genotype HUR 109 with 15.68, 19.30 and 19.35% on total chlorophyll, chlorophyll A and chlorophyll B, respectively. The observation was depicted in Table 4.6.

Interaction effect of different cultivars and lead nitrate levels found to be non-significant.

Table 4.6: The effect of lead levels on chlorophyll content (µg g^{-1} FW) in rice (*Oryza sativa* (L.)) genotypes

Chlorophyll content (µg g^{-1} FW)					
Treatments	**V_1**	**V_2**	**V_3**	**V_4**	**V_5**
L_1	134.30	132.48	136.73	125.30	116.30
L_2	106.78	88.34	99.41	97.78	88.78
L_3	103.27	83.95	89.06	94.27	85.27
Chlorophyll A					
Treatments	**V_1**	**V_2**	**V_3**	**V_4**	**V_5**
L_1	44.78	44.08	45.72	41.31	37.84
L_2	34.17	27.06	31.33	30.70	27.23
L_3	29.19	21.74	23.71	25.72	22.25
Chlorophyll B					
Treatments	**V_1**	**V_2**	**V_3**	**V_4**	**V_5**
L_1	48.46	40.69	50.72	45.19	40.10
L_2	41.85	34.01	39.37	38.58	33.49
L_3	38.95	30.68	33.16	35.68	30.59

Treatments	**Total chlorophyll content**	**Chlorophyll A**	**Chlorophyll B**
Rice cultivars			
V_1: HUR 1304	38.26	12.02	14.36
V_2: HUR 36	33.86	10.32	11.71
V_3: HUR 3022	36.13	11.20	13.69
V_4: HUR 1309	35.26	10.86	13.27
V_5: HUR 109	32.26	9.70	11.58
SEm±	1.92	0.67	1.04
LSD (p=0.05)	3.92	1.37	2.13
Lead nitrate			
L_0: Control	43.01	14.25	15.01
L_1: 2 mM	32.07	10.03	12.49
L_2: 4 mM	30.39	8.17	11.27
SEm±	1.49	0.52	0.81
LSD (p=0.05)	3.04	1.06	1.65
Interaction (V x L)	NS	NS	NS

4.2.2 Carotenoids (µg g^{-1} FW)

Carotenoids (µg g^{-1} FW) showed significant differences among five genotypes under the influence of lead nitrate levels as presented in Table 4.7. Among treatment significantly higher carotenoids (16.49 µg g^{-1} FW) was found in control, whereas the least carotenoids (11.30 µg g^{-1} FW) was observed in treatment L_2 (Pb $(NO_3)_2$ @ 4 mM). Among genotypes, significant differences in carotenoids (µg g^{-1} FW) was recorded and HUR 1304 showed significantly higher carotenoids (14.49 µg g^{-1} FW) than other genotypes in control condition. In all genotypes, L_2 showed the maximum reduction in carotenoids (11.30 µg g^{-1} FW) as compared to control. The maximum reduction in number of carotenoids (12.24 µg g^{-1} FW) was recorded in HUR 109 with 15.49 percent.

Interaction effect of different cultivars and lead nitrate levels found to be non-significant.

4.2.3 Membrane stability index (%)

Membrane stability index showed significant differences among five genotypes under the influence of lead nitrate levels as presented in Table 4.7. Among treatment significantly higher membrane stability index (77.18%) was found in control, whereas the least membrane stability index (67.86%) was observed in treatment L_2 (Pb $(NO_3)_2$ @ 4 mM). Among genotypes, significant differences in membrane stability index was recorded and HUR 1304 showed significantly higher membrane stability index (78.07%) than other genotypes in control condition. In all genotypes, L_2 showed the maximum reduction in membrane stability index (67.86%) as compared to control. The maximum reduction in membrane stability index (66.86%) was recorded in HUR 109 with 14.35 percent.

Interaction effect of different cultivars and lead nitrate levels found to be non-significant.

Table 4.7: Carotenoids (µg g^{-1} FW) and membrane stability index (%) of rice cultivars as influenced by lead nitrate

Carotenoids (µg g^{-1} FW)					
Treatments	**V_1**	**V_2**	**V_3**	**V_4**	**V_5**
L_1	51.20	43.58	58.09	50.13	44.46
L_2	41.70	36.75	41.77	40.63	34.96
L_3	37.53	31.97	32.84	36.46	30.79
Membrane stability index (%)					
Treatments	**V_1**	**V_2**	**V_3**	**V_4**	**V_5**
L_1	245.22	215.94	257.70	227.20	211.57
L_2	230.50	199.69	216.84	212.48	196.85
L_3	226.92	193.77	195.02	208.90	193.27

Treatments	**Carotenoids (µg g^{-1} FW)**	**Membrane stability index (%)**
Rice cultivars		
V_1: HUR 1304	14.49	78.07
V_2: HUR 36	12.47.	67.71
V_3: HUR 3022	14.74	74.40
V_4: HUR 1309	14.13	72.07
V_5: HUR 109	12.24	66.86
SEm±	0.22	3.68
LSD (p=0.05)	0.46	7.51
Lead nitrate		
L_0: Control	16.49	77.18
L_1: 2 mM	13.05	70.43
L_2: 4 mM	11.30	67.86
SEm±	0.17	2.85
LSD (p=0.05)	0.35	5.82
Interaction (V x L)	NS	NS

4.2.4 Total soluble sugar (mg g^{-1} FW)

Total soluble sugar showed significant differences among five genotypes under the influence of lead nitrate levels as presented in Table 4.8. Among treatment significantly higher total soluble sugar (39.00 mg g^{-1} FW) was found in L_2 (Pb $(NO_3)_2$ @ 4 mM), whereas the least total soluble sugar was observed in control. Among genotypes, significant differences in total soluble sugar was recorded and HUR 109 showed significantly higher sucrose (37.49 mg g^{-1} FW) than other genotypes in L_2 (Pb $(NO_3)_2$ @ 4 mM) condition. In all genotypes, L_2 showed the maximum increase in total soluble sugar as compared to control. The maximum increase in total soluble sugar (37.49 mg g^{-1} FW) was recorded in HUR 109 with 18.78 percent.

Interaction effect of different cultivars and lead nitrate levels found to be non-significant.

4.2.5 Soluble protein content (µg g^{-1} FW)

Soluble protein content showed significant differences among five genotypes under the influence of lead nitrate levels as presented in Table 4.8. Among treatment significantly higher soluble protein (68.71 µg^{-1} FW) was found in L_2 (Pb $(NO_3)_2$ @ 4 mM), whereas the least soluble protein was observed in control. Among genotypes, significant differences in soluble protein was recorded and HUR 109 showed significantly higher soluble protein (66.24 µg^{-1} FW) than other genotypes in L_2 (Pb $(NO_3)_2$ @ 4 mM) condition. In all genotypes, L_2 showed the maximum increase in soluble protein as compared to control. The maximum increase in soluble protein (66.24 µg g^{-1} FW) was recorded in HUR 109 with 42.54 percent.

Interaction effect of different cultivars and lead nitrate levels found to be non-significant.

Table 4.8: Total soluble sugar (mg g^{-1} FW) and soluble protein (µg g^{-1} FW) of rice cultivars as influenced by lead nitrate

Total soluble sugar (mg g^{-1} FW)					
Treatments	**V_1**	**V_2**	**V_3**	**V_4**	**V_5**
L_1	75.22	88.93	93.63	85.08	93.01
L_2	99.06	112.03	104.84	108.93	116.85
L_3	109.72	121.55	106.68	119.58	127.51
Soluble protein (µg g^{-1} FW)					
Treatments	**V_1**	**V_2**	**V_3**	**V_4**	**V_5**
L_1	93.40	138.60	135.35	124.94	146.52
L_2	145.90	189.66	163.31	177.44	208.30
L_3	178.97	220.53	179.25	210.51	241.37

Treatments	**Total soluble sugar (100 mg g^{-1})**	**Soluble protein (µg g^{-1} FW)**
Rice cultivars		
V_1: HUR 1304	31.56	46.47
V_2: HUR 36	35.83	60.98
V_3: HUR 3022	33.91	53.10
V_4: HUR 1309	34.84	56.99
V_5: HUR 109	37.49	66.24
SEm±	1.78	3.70
LSD (p=0.05)	3.63	7.55
Lead nitrate		
L_0: Control	29.06	42.59
L_1: 2 mM	36.11	58.97
L_2: 4 mM	39.00	68.71
SEm±	1.38	2.86
LSD (p=0.05)	2.81	5.85
Interaction (V x L)	NS	NS

4.2.7 Alpha amylose content (mg maltose g^{-1} h^{-1} FW)

Alpha amylose content showed significant differences among five genotypes under the influence of lead nitrate levels as presented in Table 4.9. Among treatment significantly higher alpha amylose (48.56 mg maltose g^{-1} h^{-1} FW) was found in L_2 (Pb $(NO_3)_2$ @ 4 mM), whereas the least alpha amylose was observed in control. Among genotypes, significant differences in alpha amylose was recorded and HUR 109 showed significantly higher alpha amylose (48.86 mg maltose g^{-1} h^{-1} FW) than other genotypes in L_2 (Pb $(NO_3)_2$ @ 4 mM) condition. In all genotypes, L_2 showed the maximum increase in alpha amylose as compared to control. The maximum increase in alpha amylose (48.86 mg maltose g^{-1} h^{-1} FW) was recorded in HUR 109 with 13.02 percent.

Interaction effect of different cultivars and lead nitrate levels found to be non-significant.

4.2.8 Catalase content (EU mg^{-1} protein min^{-1})

Catalase showed significant differences among five genotypes under the influence of lead nitrate levels as presented in Table 4.9. Among treatment significantly higher catalase (11.847 EU mg^{-1} protein min^{-1}) was found in L_2 (Pb $(NO_3)_2$ @ 4 mM), whereas the least catalase was observed in control. Among genotypes, significant differences in catalase was recorded and HUR 109 showed significantly higher catalase (125.17 EU mg^{-1} protein min^{-1}) than other genotypes in L_2 (Pb $(NO_3)_2$ @ 4 mM) condition. In all genotypes, L_2 showed the maximum increase in catalase as compared to control. The maximum increase in catalase (125.17 EU mg^{-1} protein min^{-1}) was recorded in HUR 109 with 44.90 percent.

Interaction effect of different cultivars and lead nitrate levels found to be non-significant.

Table 4.9: Alpha amylase (mg maltose g^{-1} h^{-1} FW) and catalase (EU mg^{-1} protein min^{-1}) of rice cultivars as influenced by lead nitrate

Alpha amylase (mg maltose g^{-1} h^{-1} FW)					
Treatments	**V_1**	**V_2**	**V_3**	**V_4**	**V_5**
L_1	112.35	123.32	128.48	115.92	131.82
L_2	134.28	144.36	135.14	137.86	151.91
L_3	142.48	151.19	132.68	146.06	156.03
Catalase (µg g^{-1})					
Treatments	**V_1**	**V_2**	**V_3**	**V_4**	**V_5**
L_1	205.82	304.53	255.72	271.60	327.24
L_2	270.09	367.06	290.32	335.86	387.91
L_3	301.49	395.80	301.03	367.27	411.38

Treatments	**Alpha amylase (mg g^{-1})**	**Catalase (EU mg^{-1} protein min^{-1})**
Rice cultivars		
V_1: HUR 1304	43.23	86.38
V_2: HUR 36	46.54	118.60
V_3: HUR 3022	44.03	94.12
V_4: HUR 1309	44.43	108.30
V_5: HUR 109	48.86	125.17
SEm±	1.63	4.19
LSD (*p*=0.05)	3.34	8.55
Lead nitrate		
L_0: Control	40.79	90.99
L_1: 2 mM	46.90	110.08
L_2: 4 mM	48.56	118.47
SEm±	1.27	3.24
LSD (*p*=0.05)	2.59	6.62
Interaction (V x L)	NS	NS

4.2.9 Super oxide dismutase content (ug g^{-1} x 10^2 g^{-1} FW min^{-1})

Super oxide dismutase showed significant differences among five genotypes under the influence of lead nitrate levels as presented in Table 4.10. Among treatment significantly higher super oxide dismutase (236.39 ug g^{-1} x 10^2 g^{-1} FW min^{-1}) was found in control, whereas the least super oxide dismutase was observed in L_2 (Pb $(NO_3)_2$ @ 4 mM). Among genotypes, significant differences in super oxide dismutase was recorded and HUR 1304 showed significantly higher super oxide dismutase (222.97 ug g^{-1} x 10^2 g^{-1} FW min^{-1}) than other genotypes in control condition. In all genotypes, L_2 showed the maximum decrease in super oxide dismutase as compared to control. The maximum increase in super oxide dismutase (222.97 ug g^{-1} x 10^2 g^{-1} FW min^{-1}) was recorded in HUR 1304 with 20.90 percent.

Interaction effect of different cultivars and lead nitrate levels found to be non-significant.

4.2.10 Ascorbate peroxidase content (EU mg^{-1} protein min^{-1})

Ascorbate peroxidase showed significant differences among five genotypes under the influence of lead nitrate levels as presented in Table 4.10. Among treatment significantly higher ascorbate peroxidase (1.39 EU mg^{-1} protein min^{-1}) was found in L_2 (Pb $(NO_3)_2$ @ 4 mM), whereas the least ascorbate peroxidase was observed in control. Among genotypes, significant differences in ascorbate peroxidase was recorded and HUR 109 showed significantly higher ascorbate peroxidase (1.30 EU mg^{-1} protein min^{-1}) than other genotypes in L_2 (Pb $(NO_3)_2$ @ 4 mM) condition. In all genotypes, L_2 showed the maximum increase in ascorbate peroxidase as compared to control. The maximum increase in ascorbate peroxidase (1.30 EU mg^{-1} protein min^{-1}) was recorded in HUR 109 with 29.23 percent.

Interaction effect of different cultivars and lead nitrate levels found to be non-significant.

Table 4.10: Super oxide dismutase (ug g^{-1} x 10^2 g^{-1} FW min^{-1}) and ascorbate peroxidase (EU mg^{-1} protein min^{-1}) of rice cultivars as influenced by lead nitrate

Super oxide dismutase (ug g^{-1} x 10^2 g^{-1} FW min^{-1})					
Treatments	**V_1**	**V_2**	**V_3**	**V_4**	**V_5**
L_1	767.83	693.16	760.93	696.02	627.85
L_2	652.42	525.59	614.78	580.61	512.44
L_3	586.45	456.86	527.35	514.64	446.46
Ascorbate peroxidase (EU mg^{-1} protein min^{-1})					
Treatments	**V_1**	**V_2**	**V_3**	**V_4**	**V_5**
L_1	1.89	2.77	2.16	2.40	2.85
L_2	2.78	3.54	2.99	3.45	3.97
L_3	3.59	4.36	3.65	4.37	4.90

Treatments	**Super oxide dismutase (ug g^{-1} x 10^2 g^{-1} FW min^{-1})**	**Ascorbate peroxidase (EU mg^{-1} protein min^{-1})**
Rice cultivars		
V_1: HUR 1304	222.97	0.92
V_2: HUR 36	186.18	1.18
V_3: HUR 3022	211.45	0.98
V_4: HUR 1309	199.03	1.13
V_5: HUR 109	176.31	1.30
SEm±	5.46	0.11
LSD (*p*=0.05)	11.14	0.23
Lead nitrate		
L_0: Control	236.39	0.80
L_1: 2 mM	192.39	1.11
L_2: 4 mM	168.78	1.39
SEm±	4.23	0.09
LSD (*p*=0.05)	8.63	0.18
Interaction (V x L)	NS	NS

CHAPTER-V

DISCUSSION

In the present chapter, an attempt has been made to explain the results of the experiment **"Effect of Lead Levels on Germination attributes and Biochemical Parameters in Rice (*Oryza sativa* L.)"** during *kharif* season of 2019 at Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh.

5.1 Effect of cultivars

Morphological parameters of rice failed to show any significant due to cultivars. However, biochemical properties of rice *viz*., chlorophyll content, carotenoids, membrane stability index, sucrose, soluble protein, alpha amylase, catalase, super oxide mutase and ascorbate peroxidase were significantly influenced due to rice cultivars.

HUR 1304 cultivar recorded significantly higher chlorophyll content, membrane stability index and super oxide dismutase which were superior over rest of the cultivars. However, HUR 3022 recorded significantly higher carotenoids content, which was statistically at par with HUR 1304 over rest of the cultivars. Among the rice cultivars, HUR 109 recorded significantly higher sucrose, soluble protein, alpha amylose, catalase content and ascorbate peroxidase content which were superior over rest of the cultivars.

In a few varieties' growth parameters observed were suppressed differentially at all lead treatments. In some varieties, it has been found that many properties are promoted differently in some lead treatments. Similar to the results determined in this study, Islam *et al.*, (2007) reported that at higher concentrations, lead accelerates germination and also causes adverse effects on the length of the radicle and hypocotyl in *Elsholtzia argyi*.

Sethy and Ghosh (2013) and Pourrut *et al.* (2011) have been reported that lead has strongly affected the seed morphology and physiology, and therefore it inhibits germination. This may be due to the interaction of lead with protease and amylase enzymes that cause germination inhibition (Amin *et al.*, 2018; Sengar *et al.*, 2008). However, as can be seen in this study, this may differ among the plant species. Thus legumes containing soybeans have been reported to be tolerant to lead levels compared to many other plant species (Alexander *et al.*, 2006; Gupta *et al.*, 2013).

5.2 Effect of lead nitrate

Morphological parameters of rice *viz*., root length, shoot length, dry matter plant^{-1} and number of leaves plant^{-1} caused significant difference due to cultivars. However, biochemical properties of rice *viz*., chlorophyll content, carotenoids, membrane stability index, sucrose, soluble protein, alpha amylase, catalase, super oxide mutase and ascorbate peroxidase were significantly influenced due to rice cultivars.

Among the lead nitrate levels, control recorded significantly higher root length, shoot length, dry matter plant^{-1} and number of leaves plant^{-1} over rest of the treatments. The decrease in seed germination percent of rice genotypes can be attributed to the slow breakdown of stored food reserves in seed by the application of lead. Under lead stress condition, alterations in the selective cell membrane permeability qualities or effects on the activity of seed enzymes like amylases and proteases that result in a decrease in the rate of food supply to the expanding plumule and radicle are other causes of reduced seed germination. The reduction in seed germination due to heavy metal treatments is in conformity with the findings of other workers (Mehboob *et al*., 2018; Shafiq *et al.,* 2008)

Among the different levels of lead nitrate, significantly higher chlorophyll content, carotenoids, membrane stability index was noted under control treatment which was superior over lead nitrate of 2 mM and 4 mM, respectively. However, 4 mM recorded significantly higher sucrose content, soluble protein, alpha amylose, super oxide dismutase and ascorbate peroxidase which was superior over rest of the treatments.

This response had already been observed by Sheng *et al*. (2005), who stated that lead significantly reduces the root length and shoot length of rice seedlings, and that the degree of inhibition increased with the increase of Pb concentration. The primary effect of Pb toxicity in plants is a rapid inhibition of root growth, probably due to the inhibition of cell division in the root tip (Eun *et al*., 2000). The reduction in root growth by Pb toxicity is most possibly from the result of a non-selective suppression of both cell division and cell elongation of the seedlings (Eun *et al*., 2000). The small and poorly developed root system of rice seedlings at elevated Pb concentrations might also be associated to a disorder of metabolic processes (Obroucheva *et al*., 1998). Lead stress negatively affected the dry weight with increasing Pb application (Kibria *et al*., 2010). The decreased in dry biomass of rice seedlings might be due to interference of Pb with the physiological processes of the plant, as Lead phytoxicity involves the decrease of enzyme activities, disturbed mineral nutrition, water imbalance and alteration in hormonal status and

variation in membrane permeability (Sharma and Dubey, 2005). The decline of biomass by Pb toxicity could be the direct consequences of the inhibition of chlorophyll synthesis and photosynthesis (Chatterjee *et al*., 2004).

This might be due to decreased morphological characters in rice due to Pb could possibly be attributed to the interference of Pb with the metabolic and biochemical processes associated with normal growth and development of the plant. Our studies on Pb uptake indicated an increased uptake of Pb in rice seedlings with increase in Pb concentration in the growth medium and that the absorbed lead is distributed in an organ specific manner with its localization greater in roots than in shoots. It has been shown that Pb is unevenly distributed in roots, where different root tissues act as barriers to apoplastic and symplastic Pb transport and hence Pb transport to shoot gets restricted (Trivedi and Erdei, 1992).

Our results indicated that in 15 day grown rice seedlings, the accumulated Pb level was higher than the Pb supplied in the growth medium. This suggests that rice, a partially halophytic plant, accumulates Pb against the concentration gradient. These results corroborate with our earlier findings with the heavy metal Cd which was also found to accumulate in rice seedlings to a greater extent in roots than in shoots and that the uptake of Cd was against concentration gradient (Shah *et al*., 2001). Increasing concentration of lead from 2 mM $(PbNO_3)_2$ to 4 mM $(PbNO_3)_2$ caused a progressive reduction in the observed photosynthetic pigments i.e., Chlorophyll a, Chlorophyll b and total Chlorophyll in all the five varieties of rice. Similar finding were obtained by Ashraf *et al*. (2017) on five aromatic rice cultivars: Faizan, S. (2011). According to earlier research, the pigment system of plant metabolism is inhibited when there is a significant quantity of lead present in the plant tissues. (Pandey and Sengar, 1996) Usually, lead accumulation in plants affects the amount of chlorophyllase, an enzyme that adversely impacts (degrades) the amount of chlorophyll (Hu *et al.,* 2012).

The present study suggests that Pb toxicity in situ leads to production of lipid peroxides and induces some of the key enzymes of antioxidant defense system in rice plants. Induction in the activities of antioxidative enzymes is a general strategy adopted by plants to overcome oxidative stress due to the imposition of environmental stresses (Malecka *et al*., 2001). Also Israr*et al.* (2011) found lead induced oxidative stress in *Sesbania drummondii.*

Catalase is universally present oxido-reductase that decomposes H_2O_2 to water and molecular oxygen and it is one of the key enzymes involved in removal of toxic peroxides (Lin and Kao, 2000). A decline in catalase activity under Pb toxicity was observed in our studies which suggests a possible delay in removal of H_2O_2 and toxic peroxides mediated by catalase and in turn an enhancement in the free radical mediated lipid peroxidation under Pb toxicity. Similar decline in catalase activity was reported under salinity, chilling, drought and hypoxia (Ushimaru *et al*., 1992).

CHAPTER-VI

SUMMARY AND CONCLUSION

The present investigation entitled **"Effect of Lead Levels on Germination attributes and Biochemical Parameters in Rice (*Oryza sativa* L.)"** was conducted during *kharif* season 2019 at Department of Plant physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh. The alarming situation of lead contamination in the southeast Asia had challenged the food and nutritional security of the ever-increasing human population. Lack of research works in this field particularly in the field of rice production had driven me to carry out the present research work. The present investigation was carried out on five rice cultivars HUR 1304, HUR 36, HUR 3022, HUR 1309 and HUR 109 with three lead nitrate levels *viz*, control (0 mM), 2 mM and 4 mM and replicate thrice. Rice were grown inside the pot. The effect of lead nitrate on plant morpho-physiological and biochemical parameters were investigated in the presence and absence of lead nitrate.

Salient findings from the present investigation were given below

1. The germination percentage of rice seeds was highest in control, whereas the least per cent germination was observed in treatment L_2 (Pb $(NO_3)_2$ @ 4 mM). Among genotypes, higher germination (93.96%) was noted under HUR 36 cultivar.
2. The radicle and plumule length were found longest in control, whereas the shortest length was recorded in treatment L_2 (Pb $(NO_3)_2$ @ 4 mM) at both stages. Among genotypes, HUR 1304 showed higher radicle and plumule length than other genotypes in control treatment at both the stages.
3. Higher dry matter seedling^{-1} and number of leaves seedling^{-1} were found in control, whereas the least dry matter seedling^{-1} was recorded in treatment L_2 (Pb $(NO_3)_2$ @ 4 mM) at both stages. Among genotypes, HUR 1304 showed higher dry matter seedling^{-1} than other genotypes in control treatment at both the stages.
4. Among treatments, higher chlorophyll content was found in control, whereas the least chlorophyll content was recorded in treatment L_2 (Pb $(NO_3)_2$ @ 4 mM) on chlorophyll A, B and total chlorophyll. Among genotypes, significant differences in chlorophyll

content were observed. HUR 1304 showed significantly higher chlorophyll content than other genotypes in control treatment on chlorophyll A, B and total chlorophyll.

5. Significantly higher carotenoids (16.49 µg g^{-1} FW) and membrane stability index (77.18%) were found in control, whereas the least carotenoids (11.30 µg g^{-1} FW) was observed in treatment L_2 (Pb $(NO_3)_2$ @ 4 mM). Among genotypes, significantly higher carotenoids (14.49 µg g^{-1} FW) was noted under HUR 1304 cultivar than other genotypes in control condition.
6. Significantly higher total soluble sugar (39.00 mg g^{-1} FW) was found in L_2 (Pb $(NO_3)_2$ @ 4 mM), whereas the least under control. Among genotypes, HUR 109 showed significantly higher sucrose (37.49 mg g^{-1} FW) than other genotypes in L_2 (Pb $(NO_3)_2$ @ 4 mM) condition.
7. Among treatment significantly higher soluble protein (68.71 μg^{-1}) and alpha amylose (48.56 mg g^{-1}) were found in L_2 (Pb $(NO_3)_2$ @ 4 mM), whereas the least soluble protein was observed in control. Among genotypes, HUR 109 showed significantly higher total soluble protein (66.24 μg^{-1}) than other genotypes in L_2 (Pb $(NO_3)_2$ @ 4 mM) condition.
8. Significantly higher catalase (11.847 EU mg^{-1} protein min^{-1}) was found in L_2 (Pb $(NO_3)_2$ @ 4 mM), whereas the least catalase was observed in control. Among genotypes, HUR 109 showed significantly higher catalase (125.17 EU mg^{-1} protein min^{-1}) than other genotypes in L_2 (Pb $(NO_3)_2$ @ 4 mM) condition.
9. Among treatment significantly higher super oxide dismutase (236.39 ug g^{-1} x 10^2 g^{-1} FW min^{-1}) was found in control, whereas the least super oxide dismutase was observed in L_2 (Pb $(NO_3)_2$ @ 4 mM). Among genotypes, HUR 1304 showed significantly higher super oxide dismutase (222.97 ug g^{-1} x 10^2 g^{-1} FW min^{-1}) than other genotypes in control condition.
10. Significantly higher ascorbate peroxidase (1.39 EU mg^{-1} protein min^{-1}) was found in L_2 (Pb $(NO_3)_2$ @ 4 mM), whereas the least ascorbate peroxidase was observed in control. Among genotypes, HUR 109 showed significantly higher ascorbate peroxidase (1.30 EU mg^{-1} protein min^{-1}) than other genotypes in L_2 (Pb $(NO_3)_2$ @ 4 mM) condition.

Conclusion

From the present investigation it has been concluded that the early seedling growth of rice crop was sensitive to lead nitrate toxicity, which was evident in the form of reduction in germination, radicle and plumule growth, dry matter and leaves seedling^{-1}. Induced lead nitrate toxicity causes reduction in different morphological and biochemical parameters *viz*. radicle and plumule length, dry matter seedling^{-1}, no of leaves, chlorophyll content, carotenoid and membrane stability index however, it increased the concentration of lead nitrate in rice crop. Application of lead nitrate had beneficial effect on total soluble solids, soluble protein, alpha amylase, catalase, super oxide dismutase and ascorbate peroxidase. In case of cultivars, HUR 3022 was best under lead nitrate toxicity effect in terms of radicle length, plumule length, dry matter seedling^{-1}, number of leaves seedling^{-1} and carotenoids. However, HUR 1304 cultivar showed superiority for germination, chlorophyll content, membrane stability index and super oxide dismutase over rest of the cultivars. While, in case of total soluble sugar, soluble protein, alpha amylase, catalase and ascorbate peroxidase were significantly improved with cultivar HUR 109.

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