EFFECT OF NATRIUM FLUORIDE ON GROWTH AND PHYSIOLOGY OF *ORYZA SATIVA* L.

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BY

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CERTIFICATE

I declare that the dissertation entitled “EFFECT OF NATRIUM FLUORIDE ON GROWTH AND PHYSIOLOGY OF *ORYZA SATIVA* L.” has been prepared by me under the guidance of Prof. P. Ramarao, Administrative guide, Acting Dean, School of Environment and Earth Sciences and Dr. Sunil Mittal, Assistant Professor, Centre for Environmental Science and Technology, Central University of Punjab. No part of this dissertation has formed the basis for the award of any degree or fellowship previously.

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CERTIFICATE

We certify that ANAMIKA DAS has prepared her dissertation entitled ‘‘EFFECT OF NATRIUM FLUORIDE ON GROWTH AND PHYSIOLOGY OF *ORYZA SATIVA* L.”, for the award of M.Phil. degree of the Central University of Punjab, under ourguidance. She has carried out this work at the Centre for Environmental Science and Technology, School of Environment and Earth Sciences, Central University of Punjab.

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ABSTRACT

Effect of Natrium Fluoride on Growth and Physiology of *Oryza sativa* L.

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Key words	: Natrium Fluoride Toxicity; Reactive Oxygen Species; Superoxide Dismutase; PR147; Basmati 1121.

The present study was undertaken to investigate the effect of natrium fluoride on growth and physiology of *Oryza sativa* L. Two varieties of the *O. sativa* (rice) viz. Basmati 1121 and PR147 were selected for the study. PR147 is a local variety of the region. The effect was studied on growth (root length and shoot length), physiological parameters (chlorophyll content and percent respiration), macromolecules and their hydrolyzing enzymes (carbohydrate and protein content, α-amylase and protease activity) and antioxidant enzymes (peroxidase and superoxide dismutase) under controlled conditions in the laboratory. Studies revealed that NaF (in different concentrations ranging from 16, 32 and 64 mg/l) affected the growth and physiology of both varieties as compared to control. When the seed of both varieties of rice were exposed to 64 mg/l NaF concentration, the seedling growth of test plants was highly affected as compared to control. Basmati 1121 variety was found to be more sensitive than PR147. In chlorophyll content, the percent reduction at 64 mg/l of NaF was 62% in Basmati 1121 and 30% in PR147. Similarly, reduction in percent cellular respiratory ability on exposure to 64 mg/l of NaF was 75% and 22% in Basmati 1121 and PR147 variety respectively. At 64 mg/l the carbohydrate content was increased 2.9 folds in Basmati 1121 and 1.35 folds in PR147. However the protein content was variably affected as it was increased 2 folds in PR147 and decreased to nearly 55% in Basmati 1121. The macromolecular contents were affected as the activity of their hydrolyzing enzymes was also affected. The increase in oxidative stress due to high concentrations of fluoride exposure has lead to an increase in antioxidant enzyme activity of both peroxidase and superoxide dismutase enzymes. The study concludes that high content of fluoride affects the growth and physiology of *O. sativa* L. by inducing oxidative stress.

(Anamika Das) (Dr. Sunil Mittal) (Prof. P. Ramarao)

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LIST OF ABBREVIATIONS

Sr.No.	Full form	Abbreviation
1.	Adenosine triphosphate	ATP
2.	Reactive oxygen species	ROS
3.	Superoxide dismutase	SOD
4.	World Health Organization	WHO
5.	Thin layer chromatography	TLC
6.	Revolutions per minute	RPM
7.	Di-methyl sulphoxide	DMSO
8.	Triphenyl tetrazolium chloride	TTC
9.	Ethylene diammine tetraacetic acid	EDTA
10.	Trichloro acetic acid	TCA

CHAPTER-1

INTRODUCTION

Fluorine has been reported as the thirteenth most abundant element present in the earth. Fluorine being the most chemically reactive and electronegative of all the elements and forms number of compounds. Compounds containing fluorine anions are called fluorides.

Fluorine exists as fluoride bounded to both organic and inorganic cations. Fluorides exist in nature in inert form such as calcium fluoride to highly reactive forms such as sulfur tetra fluoride. The main sources of fluoride in nature are weathering of rocks, emissions from volcanoes, sea water etc. The anthropogenic sources like mining of phosphate rocks, phosphate fertilizers in agricultural fields and fluoridation of drinking-water supplies also adds fluoride in soil and water.

Fluorides are taken up by plants from soil and water. However, its uptake depends on various factors like soil profile, organic content and soil pH. Fluoride ions are more soluble in acidic soils and more tightly bound in clay soil. Hence, the absorption of fluoride ions by plants is more in acidic sandy soils compared to alkaline clay soils. From plants, the fluorides may be passed into the animals including humans through food chain. Drinking water with high fluorides is another source of fluoride in animals. The fluoride is present in bones, teeth and many soft tissues of humans and animals.

However, high content of fluoride causes many health problems to human beings and domestic animals. The primary effects of fluoride manifestation include skeletal mottling like dental fluorosis and osteosclerosis apart from non-skeletal fluorosis or toxic effects of fluoride in soft tissues, like gastro-intestinal discomforts, neurological disorders, impaired reproductive functions and teratogenic effects (Choubisa, 1999; Choubisa, 2008).

Fluoride has diverse effects on plants depending upon its concentration (Weinstein, 1977). As the concentration of fluoride increases it causes severe effects on the plants. It causes physiological and biochemical changes in the plant. Fluoride affects the plant physiology by affecting the seedling growth, total chlorophyll content and

percent cellular respiratory ability etc. Biochemically, it affects the enzymatic activity, carbohydrate content, protein content etc.

Fluoride affects the seedling growth by affecting the root length and shoot length of the seedling (Bhargava and Bhardwaj, 2010; Gupta *et al.,* 2009; Sabal *et al.,* 2006; Sarkar *et al.,* 1982). Chlorophyll is the green pigment which carries out the process of photosynthesis required to obtain energy in plants. The chlorophyll pigment gets affected due to the presence of fluoride and the fluoride concentration reduces the total chlorophyll content (Gupta *et al.,* 2009; Elloumi *et al.,* 2005; McNulty and Newman, 1961).

Respiration is an important process consists of various metabolic reactions required for the conversion of biochemical energy from nutrients into ATP. Fuelling of cellular reformations is done during respiration. The total respiratory ability of the plant is exhibited by green leaves. Fluoride can inhibit the respiratory rate in plants (Oridin and Jacobson, 1955; Applegate *et al*., 1960; McNulty and Lords, 1960; Miller and Miller, 1974).

Fluoride concentration not only affects the physiological activity of the plant but also affects its biochemical activity. Fluoride is able to inhibit the protein, lipid and carbohydrates metabolisms. The inhibition mechanism is not clearly understood, but the enzymes requiring cofactors such as Ca^{2+}, Mg^{2+} and Mn^{2+} ions, are inhibited by fluoride at high concentration. For example, amylase (Yu *et al.,* 1988) and invertase (Yu, 1997) were inhibited in germinating mung bean seedlings by sodium fluoride (natrium fluoride) and protease enzymes were inhibited in rice and jute seedlings (Sarkar *et al*., 1982). Amylases are required for the breakdown of polysaccharide starch into smaller sugar chains like maltose. They are mainly found in seeds of cereals like wheat, rice etc. Germinating seeds are the storehouse for amylases. They require amylases for the conversion of complex chain of starch in the endosperm into simpler or more usable forms.

According to Schaller (2004), all important aspects of plants development require the breakdown of proteins and this is done by plant proteases. The protein turnover (balance between protein synthesis and protein degradation) is concerned with protease activity by removing damaged proteins from cells. Modifying proteins are required for definite function in plants and assist enzymes to mature. Different proteases have different functions in plant like cysteine

proteases are responsible for seed germination and programmed cell death while serine proteases is required for protein turnover.

Fluorides are also responsible for affecting antioxidant activity of the enzymes. Likewise, when the cellular homeostasis gets disrupted, level of reactive oxygen species (ROS) increases which is harmful for living cells. Due to these ROS disruption of normal metabolism of living cells occur by disrupting lipids, nucleic acids and proteins (Mckerise and Ya'acov, 1994). Increase in ROS triggers the production of antioxidant enzymes. In plants for the occurrence of normal metabolism efficient removal of reactive oxygen species is required.

In aerobic cells, scavenging systems for reactive oxygen species include superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase and they are responsible for the detoxification of reactive oxygen species (Alscher *et al.*, 1997).

Superoxide dismutase enzymes play a key role in higher plants by acting as an antioxidant and protecting cellular components from oxidation due to reactive oxygen species (ROS). Reduction of molecular O_2 to O^{2-} (superoxide ion) occurs when an excited electron released from compounds of the electron transport chain gets absorbed by O_2 and the superoxide ion denatures enzymes, oxidize lipids, and fragment DNA (Smirnoff, 1993). So the superoxide dismutase catalyzes the generation of O_2 and H_2O_2 from superoxide (O^{2-}), which results in less harmful reactants. According to Wilde and Yu (1998), there is a significant increase in the SOD activity in germinating mung bean seedlings after treating with fluoride.

During stress conditions the total concentration of SOD increases. There are basically three types of SOD metallic coenzymes exist in plants. One is Fe SOD which consists of homodimer (containing 1-2 g Fe) and one tetramer of (containing 2-4 g Fe). Second one is Mn SOD mainly found in mitochondria and peroxisomes and the third one is Cu-Zn SOD having electrical properties found in chloroplast, cytosol, and in some cases the extracellular space (Alscher *et al.*, 2002). It is the most primitive SOD metalloenzymes and found in both prokaryotes and eukaryotes. They are plentiful within the plant chloroplasts and are of indigenous nature (Raychaudhuri and Deng, 2000).

Peroxidases are the oxido-reductases which oxidizes molecules by utilizing hydrogen peroxide and they belong to the family of isozymes found in all plants. They contain heme group and are monomeric in nature (Yoshida *et al.*, 2003). Plants have an active defense mechanism against pathogenic substances. A rapid increase in peroxidase concentration has been observed in plants inoculated with selected viruses, bacteria or fungi and frequent emergence of new peroxidase isozymes (Stahmann and Demorest, 1973). Fluoride sensitivity amid numerous species and the species of different cultivars varies (Posthumus, 1983).

The present study concentrates on the effect of fluoride on two varieties of rice. Rice (*Oryza sativa*) belongs to the family Poaceae and India occupies the 2nd position for its production in the world. Punjab accounts 9% of the total rice production in India.

Rice is one of the important crops grown in district Bathinda as an annual plant. It is a kharif (harvested in autumn) crop. Sowing of rice crop demands plenty of water. In some areas of this region groundwater is used for irrigation purposes which have raised a serious issue regarding the transfer of fluoride through food chain, because groundwater of Bathinda district has fluoride more than the permissible limits. The permissible limit of fluoride is 1.5 mg/l according to WHO (World Health Organization).

In our present study, two varieties of rice grown in this region were taken to see the effect of fluoride on seedling growth, physiological parameters and biochemical parameters were considered. It will give us an idea about the effect of different concentrations of fluoride on rice plants. By studying two different variety of rice a comparison can be established that which variety is more tolerant to fluoride concentration.

CHAPTER-2

REVIEW OF LITERATURE

The plants get exposed to fluorides from air, water and soil. In air, most common form of fluoride pollutant is hydrogen fluoride which gets absorbed by plants through leaves. However, uptake of fluoride by plants through soil and water is more common throughout the world including India (Handa, 1975). Bathinda is one such district where the concentration of fluoride is reported high (CGWB, 2007). Fluoride in soil and water occur in many salt forms like aluminum fluoride, calcium fluoride, sodium fluoride etc. However, sodium fluoride is more common in ground water and soil.

The fluoride salts has been reported as metabolic inhibitors and affect wide range of physiological and biochemical processes (Yu *et al*., 1987; Reddy *et al*., 1989; Mathews and Holde, 1990; Reddy and Venugopal, 1990). The high concentration of fluoride causes various harmful effects to exposed plants as indicated in many studies done by various scientists (Choudhary and Bohra, 1989; Rakowski *et al*., 1995, Yu, 1997; Kumar and Rao, 2008; Pant *et al*., 2008). Both the forms of fluoride gaseous as well as water soluble form have been reported to adversely affect the plants.

2.1 Effect of fluoride on seedling growth

Water soluble forms of fluoride ion have reported to affect growth of plants. The plant growth is affected probably due to effect on physiological processes like chlorophyll, respiration, leaf tip burn etc. Many reports have been published establishing the reduction in various physiological parameters; viz., root length, shoot length and dry weight in response to high concentrations of sodium fluoride (Bhargava and Bhardwaj, 2010).

A study carried out by Sabal *et al.* (2006) on cluster bean (*Cyamopsis tetragonoloba*) seed germination and seedling growth reported negative effect of fluoride on seed germination, while the root and shoot length affected the total biomass.

Bozhkov *et al*. (2009) studied the effect of sodium fluoride on the root apex border cells (BC) in one day old wheat. NaF (1–20 mM) suppressed growth of wheat seedling roots; the viscosity of the gel sheath increased (by 3–5 times), and the

number of BC rose with the most pronounced increment in the size of the BC subpopulation directly associated with the root apex.

Yang and Miller (1963) reported the increase in concentration of three enzymes, phosphoglucomutase, uridine diphosphate glucose pyrophosphorylase and uridine diphosphate glucose-fructose transglucosylase in response to hydrogen fluoride. Similarly Reynolds and Laurence (1988) reported the inhibition in field-grown red kidney beans growth on exposure to hydrogen fluoride.

2.2 Effect of fluoride on Physiological parameters

Gupta *et al.* (2009) studied phytotoxicity of fluoride in the germination of paddy (*Oryza sativa*) and its effect on the physiology and biochemistry of germinated seedlings and they reported that physiological parameters, viz., root length, shoot length, and dry weight decreased monotonically with increasing NaF concentration and even the chlorophyll content of the leaves also decreased monotonically, but the reducing sugar and ascorbic acid content initially decreased and then increased with increasing concentration of NaF.

Reddy and Kaur (2008) studied the effect of sodium fluoride on *Salicornia brachiata* Roxb. This was the first report on fluoride tolerance in marshy halophytes using as high as 150 mM concentration and reported that photosynthetic pigments (chlorophylls and carotenoids) content decreased, while, anthocyanin content increased significantly with fluoride treatment.

Further, Elluomi *et al*. (2005) observed significant decrease in chlorophyll, starch and sugar in leaves of *Amygdalis communis* in response to higher concentrations of fluoride which ultimately lead to the decrease in the nutritional quality of the leafs. Saleh and Abdel-Kader (2003) has reported the significant decrease in pigment levels (chlorophyll a & b, total carotenoids and anthocyanins) in the two cultivars of sunflower. The changes in photosynthetic and enzyme activity caused by high fluoride exposure may lead to altered growth, development, and reproduction.

Adams and Sulzback (1961) reported that fluoride has affected the photosynthesis rates, respiration and chlorophyll destruction. A further change in chlorophyll content was observed by Rabe and Kreeb (1980).

Roberta (2001) reported the alterations in leaf micro-morphology and anatomy, together with the changes found in some biochemical parameters following experimental treatments with fluorides (NaF) in controlled conditions on *Hypericum perforatum* plants. Severe wilting occurs in mature leaves, and then the tissues of the tip and the margin leaf portions change their colour, becoming red-brownish. Among the cell organelles, the chloroplasts are greatly altered: the lamellar system is scarcely recognizable and the external envelope interrupted. Photosynthetic pigments decrease and the anthocyanin content increases. Within 72 hour of NaF treatment Superoxide dismutase activity was negatively affected.

In the year 1963, low respiratory quotient was observed by Yang and Miller in fluoride fumigated leaves. In many studies inhibition of respiration was observed due to fluoride exposure (Ordin and Jacobson, 1955; Applegate *et al*., 1960; McNulty and Lords, 1960). Fluoride causes decrease in tissue respiration which is certainly due to inhibition of respiratory enzymes viz. succinate, malate, NADH dehydrogenases (Lovelace and Miller, 1967), enolase (Miller, 1958), Phosphoglucomutase (Yang and Miller, 1963) and hexokinase (Melchior and Melchior, 1956). Studies by Bonner and Thimann, 1950 has reported that the amount of fluoride which inhibited the growth of oat coleoptiles is not enough to inhibit the respiration rates. Nickerson and Chung (1952) supported the similar results in yeast cells.

Miller and Miller, 1974 studied the effect of sodium fluoride on respiration of mitochondria and plant tissue. Stimulation of respiration occurred after 2 days when fumigated with hydrogen fluoride and 2 days later inhibition occurred. Higher respiration rates and greater ATPase activity was visible in mitochondria isolated from stimulated tissue and reduction in inhibited tissue. Mitochondrion isolated from etiolated corn shoots (*Zea mays* L. cv. Golden Cross Bantam) has increased the amount of osmotically induced swelling when treated with fluoride.

2.3 Effect of fluoride on macromolecules and their hydrolyzing enzyme

According to Li and Ni (2009), fluoride has decreased the protein concentration, polyphenols and total catechins. Except for magnesium and manganese, the uptake of most of minerals was inhibited, whereas the content of fluorides increased markedly. Finally these results suggests that due to fluoride treatment

the main chemical constituents of tea leaves decreased and suggesting that these teas consumed by the individuals may ingest excessive amounts of fluorides.

Saleh and Abdel-Kader (2003) have reported decrease in the activity of many enzymes including amylases in response to increase in concentration of fluoride. Increasing fluoride concentration has also reported to affect the photosynthetic activity of plants.

It has been reported by various scientists that the enzymes requiring cofactors such as $Ca^{2+,}$ Mg^{2+}, and Mn^{2+} ions were inhibited by fluoride viz. amylase (Yu *et al*., 1988) and invertase (Yu, 1997) in germinating mung beans. He further suggested that F^- induced inhibition of amylase in mung bean seedlings may be due to the interaction of F^- with Ca^{2+}, which is required for enzyme activity.

One of the studies done by Viola and Davies (1991) that the fluoride is responsible for the inhibition of glycolysis pathway and it also caused the stimulation of triose-phosphate recycling. Incorporation of [U-'4C] glucose, [U-14C] sucrose, [U-14C] glucose 1-phosphate, and [U-14C] glycerol into starch was also inhibited by fluoride.

2.4 Effect of fluoride on antioxidant enzymes

Among biochemical effects of fluorides, varying effects have been reported on antioxidant enzymes in different plants. These antioxidant enzymes provide defense to cells against free radicals by scavenging mechanism. Wilde and Yu (1998) have also reported, decrease in SOD activity with increasing amount of fluoride in mung seedlings. Kumar *et al*. (2009) have taken sodium fluoride to study the effect of fluoride on catalase, guiacol peroxidase and ascorbate oxidase activities in two varieties of Mulberry Leaves (*Morus alba* L.) and have reported significant alleviation in activities of these enzymes. Similar affects of high concentration of natrium fluoride on enzymatic activities of rice (*Oryza sativa* L.) and jute (*Corchorus olitorius* L.) seedlings have been reported by Sarkar *et al*. (1982). Reddy and Kaur (2008) reported that peroxidase (POx), superoxide dismutase (SOD), ATPase and acid phosphate activities were negatively regulated. In addition F^-, Na+, Mn^{2+} and Fe^{2+} ions concentration increased while, K^+, Ca^{2+} and Mg^{2+} contents decreased with fluoride treatment. The results suggests *S. brachiata* is a moderately fluoride tolerant annual halophyte.

Fluoride is also responsible to cause necrosis and chlorosis as the first visible symptoms in plants (Griffin and Bayles, 1952; Haas and Brusca, 1955; Kendrick *et al*., 1956).

Further Katz and Shore (1955) reported the degradation of chlorophyll by fluoride causing conversion to the corresponding pheophytins.

Growth response of Pinto bean and alfalfa to sublethal fluoride concentrations was studied by Treshow and Harner (1968). They found that fresh and dry plant weights were positively correlated with increasing fluoride content of the leaves when they were fumigated with the fluoride. Though, the growth of alfa-alfa was stimulated but the results were inconsistent.

Singh *et al*. (2009) observed the fluoride toxicity on chlorophyll, protein percentage and energy content of hybrid rice (*Oryza sativa* L.).

2.5 Effect of fluoride accumulation

2.5.1 On animals

In one of the studies done by Choubisa (2010) has reported skeletal fluorosis in camel. In the same study they reported osteo-dental fluorosis in domestic horses and donkeys.

2.5.2 On plants

Recent studies by Gautam *et al*. (2010) have concluded that the green leafy vegetables tend to accumulate more amount of fluoride compare to non leafy crops.

Further, Gautam and Bhardwaj (2010) reported that fluorides tend to accumulate more in roots as compared to other parts owing to its relatively low mobility.

Fluoride accumulation was also observed in different parts of the paddy plant irrigated with fluoride contaminated ground water by Gupta and Banerjee (2009). Root has been reported to accumulate the highest amount of fluoride, then leafs, stems and at last the seeds.

Singh *et al.* (1995) conducted studies on uptake of fluoride rich irrigation water by the plants. Study was carried out on Lady finger (*Abelmoschus esculentus*) and it was grown in sand and soil cultures for 18 week and the fluoride accumulation in various plant parts was studied. It was considered that food chain plays an

important role for the consumption of fluoride by human being. They observed that the sand cultured plants had accumulated greater percentage of fluoride than soil-cultured plants. Decrease in accumulation observed from root to fruit when fluoride is supplied through irrigation water. They found that Lady finger plants irrigated with water containing 10 mg/l of fluoride accumulates 0.20 mg per 100 g lady finger.

Taylor & Basabe (1984) studied a correlation between fluoride concentrations in pine needles (Douglas-fir, *Pseudotsuga menziesii*) and annual growth increments, wind pattern, distance from fluoride source (Aluminium smelter) and hydrogen fluoride concentrations in emissions. Visible fluoride symptoms and growth reduction over 40% was observed in trees by the authors and that were accumulating fluorides below established "injury threshold levels." Synergism between hydrogen fluoride and sulfur dioxide was the reason behind reduced threshold levels.

2.6 Other Effects

2.6.1 Leaf injury

Vike & Håbjørg (1995) confirmed leaf injury in a variety of plant species growing in the vicinity of Aluminium smelters in Norway and fluoride content. They reported that Scots pine (*Pinus sylvestris*) was the most sensitive species than broad-leaved species such as downy birch (*Betula pubescens*), goat willow (*Salix caprea*) and European mountain-ash (*Sorbus aucuparia*). Further, Vike (1999) reported that fluoride caused leaf injury at leaf fluoride content levels as low as 30 mg/kg, and impairment was limited to within 2 km of the emission sources. Within a locality a positive correlation between leaf injury and fluoride content of leaves was observed by regression analysis, but localities have great variation in between.

Sun and Su (1985) reported chronic symptoms of yellowing and mottling and acute symptoms of tip necrosis in Leaves of rice plants grown in the ceramic and brick industry areas of Taiwan. Increasing severity of the injury in rice leaves complemented the increase in fluoride concentration. There is a huge difference of 80 fold in fluoride concentration between severely injured and healthy leaves. New

rice disease has been proposed to be caused due to fluoride emitted from the ceramic and brick factories.

Ivinskis & Murray (1984) established that leaf fluoride content, fluoride in air and distance from an aluminium smelter were significantly correlated with the reductions in photosynthetic capacity, chlorophyll a and b and leaf area of grey gum (*Eucalyptus punctata*). No significant differences between sites for any of the variables except leaf area was confirmed in dusky-leaved ironbark (*Eucalyptus fibrosa*), a fluoride tolerant species.

There are several other effects of fluoride studied by various scientists viz. Palomaki *et al.* (1992) studied the effect of fluoride concentration on Norway spruce (*Picea abies* L. Karst) needles for biomonitoring of fluoride distribution in an area. Later, Kinnunen *et al*. (2003) analyzed the fluoride concentration in birch (*Betula Pendula* Roth) leaves, ground vegetation, litter layer and humus layer and they evaluated the validity of this plant as bio-indicator of fluoride pollution. They reported that the humus and litter layer contains highest amount of fluoride which have been accumulated over three decades.

2.6.2 Effect on cell membranes

Using light and electron microscopy techniques Zwiazek and Shay (1987) studied injuries in mesophyll and guard cells induced by fluoride and drought in jack pine (*Pinus banksiana* Lamb.) cotyledons. Similar alterations were observed in cells of fluoride and water stressed seedlings. Lipid material appeared in the cytoplasm during early stages of injury as a result of both fluoride and drought treatments. This signifies the damage to the cell membranes, while deposition of starch in chloroplasts results due to treatment with sodium fluoride. In the experiments guard cells were found more resistant than mesophyll cells to both stresses. In fluoride-treated seedlings the opening of stomata in wilting occurs due to metabolic injury and collapse of neighboring cells.

Further Zwiazek and Shay (1988) using chromatography techniques studied the effect of sodium fluoride on the lipid and fatty acid composition of jack pine (*Pinus banksiana* Lamb.) seedlings. An increase in non-polar lipids and reduction in polar lipids was observed due to fluoride treatment. There is a little effect on fatty acid composition. Reduction in behenic acid and increase in palmitic and stearic acid

were observed in number of fluoride treatments. Increase in solute leakage from cells complemented the changes in lipid and fatty acid composition.

2.6.3 Correlation of fluoride and acidity

Horner and Bell (1995) studied the relation between acidity and fluoride on early growth of *Lolium perenne* (cultivar S23) and *Triticum aestivum* (cultivar 'Perinarth') by culturing in simple solution and doing pot experiments. Synergistic interaction between acidity and fluoride was observed in Culture solution experiments. They concluded that this occurred because at low pH fluoride is in non-ionic form, hence, cell membranes readily takes it up. A significant effect of fluoride in two artificial soil mixtures was observed in pot trials, but simulated acid rain or any other interaction showed no effect. It is almost certainly due to the buffering and fluoride fixation capacities of the soils used.

2.6.4 Effect on Flavonoids

In 2009, Noori *et al.* selected six legume species for their study where effect of sodium fluoride on flavonoids was observed. In plants collected from factory area the fluoride was analyzed using ion selective electrode. Two dimensional paper chromatography (2-D PC) and TLC was used for the identification of flavonoids in the samples. The plants located 10 km distance from the factory were taken as controls. Appearance and disappearance of flavonoids is the phytochemical change observed in the polluted leaves.

2.6.5 Effect on genetic toxicity

Mohamed (1970) studied on Maize seedlings of the genotype $A_1A_2C^1Wx$ which were fumigated with hydrogen fluoride (HF) in growth chambers at a concentration of about 3 $\mu g/m^3$. 10 days experiment was established, after 4 days the first group of treated plants were removed from the chambers and then at a regular intervals of 2 days. Chromosomal aberrations were revealed in microsporocyte smears from the treated plants that included asynaptic regions, translocations, inversions, and bridges plus fragments or fragments by themselves. The physiological effect caused by HF is the stickiness of chromosomes and the occurrence of chromatid breakage and the structural changes due to reunion. The study gives indication that that HF is a mutagenic agent.

Conclusion of the review:

Although scientists have studied the effect of fluoride on various plants and on various parameters. But, very little work was done on rice plant. Scientists have studied the seedling germination and various physiological parameters on different varieties of rice. In our present study we are taking two different varieties of rice cultivated in this region and we are considering various parameters to see the effect of fluoride.

CHAPTER-3

OBJECTIVE AND PLAN OF WORK

3.1 Objective:

- To study the effect of NaF on seedling growth of two varieties of *Oryza sativa* L.
- To study the effect of NaF on physiological parameters of two varieties of *O. sativa* L.
- To study the effect of NaF on biochemical parameters of two varieties of *O. sativa* L.

3.2 Plan of work:

- Collection of seeds from Punjab Agricultural University, Ludhiana.
- Study the effect of fluoride on seedling growth in terms of
  - Root length
  - Shoot length
- Study the effect of fluoride on physiological parameters in terms of
  - Photosynthetic pigments
  - % cellular respiratory ability
- Study the effect of fluoride on macromolecules and their hydrolysing enzymes in terms of
  - Carbohydrate and α-amylase
  - Protein and protease
- Study the oxidative stress caused by natrium fluoride in terms of
  - Peroxidase activity
  - Superoxide dismutase activity

CHAPTER-4

MATERIALS AND METHODS

4.1 INSTRUMENTS

4.1.1 Electronic Weighing Balance

Sartorius Model TE64 with least measurement 0.1 mg was used for all type of weighing purposes like weighing salts to prepare solutions.

4.1.2 pH meter

The pH meter has been used to examine the pH of the solutions. The pH meter with glass electrode of water analyzer kit (Systronics) was used to adjust as well as verify the pH of the solution. Calibration of the pH meter was done at pH 4.0, 7.0 and 9.2 with buffer solutions.

4.1.3 Centrifuge

Spinwin (Feedback control Digital Timer Function) MC-02 was used for the centrifugation. It has the maximum RPM of 13500.

4.1.4 Water-bath

JSGW Rectangular water Bath with a maximum temperature of 110 °C. It has been used for heating in carbohydrate estimation and estimation of percent cellular respiratory ability.

4.1.5 Ultra Violet and Visible (UV-VIS) Spectrophotometer

Systronics UV-VIS double beam 2202 spectrophotometer was used to analyze the enzymatic activities and the total protein content, carbohydrate content, chlorophyll content, respiration ability based on different wavelength.

4.2 MATERIALS

4.2.1 Collection of seeds: The seeds of rice were collected from Punjab Agricultural University, Ludhiana. Two varieties of *O. sativa* L. were Basmati 1121 and PR147 variety. The seeds were kept in sterile conditions.

4.2.2 Element used: In this study sodium fluoride (SD-fine, mol wt. 41.99) was used to study its effect on various physiological parameters of the plants used.

4.2.3 Experiment set-up: Fifteen sterilized seeds were placed in individual petri-plates labeled as control and NaF concentrations viz. 16 mg/l, 32 mg/l, 64 mg/l, and the control. The pre-sterilized petri-plates were lined with filter paper above the moistened sterilized cotton pads. Five replicates were taken for each respective concentration of fluoride and control. On each odd day till 7 days, 5-10 ml solutions of the sodium fluoride and distilled water were added to each petri plate. The petri-plates were kept in laboratory conditions for 7 days. Root length, shoot length, chlorophyll content, total cellular respiratory ability, protein content, carbohydrate content, α-amylase activity, protease activity, SOD and peroxidase enzyme activity were studied on the 7^{th} day of the experiment. To study the chlorophyll content and cellular respiratory ability, the shoot of the seedling was used and for rest of the parameters mentioned above, the homogenate of the roots were prepared using 0.1M phosphate buffer in mortar and pestle. Supernatant was used after centrifugation done at 10,000 rpm.

4.3 METHODS

4.3.1 Chlorophyll content (Hiscox and Israelstam, 1979)

Reagent: DMSO (Dimethyl sulphoxide).

Procedure:

Took 50 mg shoot and suspended in test tubes containing 8 ml of DMSO. The test tubes were incubated at 60 ºC for one hour. The chlorophyll thus recovered in DMSO was measured at dual wavelength of 645 and 663 nm using DMSO as blank.

Calculation:

The calculation for both chlorophyll a and chlorophyll b was done on dry weight basis.

$$\text{Chlorophyll a} = (10.63 \times A_{663}) - (2.39 \times A_{645}) = X$$

$$\text{Chlorophyll b} = (20.11 \times A_{645}) - (5.18 \times A_{663}) = Y$$

$$\text{Ratio of chlorophyll a/b} = X/Y$$

Where, A_{645} and A_{663} represent extinction values at 645 nm and 663 nm, respectively.

4.3.2 Estimation of percent cellular respiratory ability (Steponkus and Lanphear, 1967)

Reagents: a) Phosphate buffer solution, pH 7.4, 0.1 M.

b) 2,3,%-Triphenyl tetrazolium chloride, TTC solution: 0.6 w/v was prepared by dissolving 600 mg of TTC in 100 ml of 0.1 M Phosphate buffer.

Method:

Took 50 mg of tissue from fully expanded leaves of test plants and dipped in test tubes containing 1.5 ml of freshly prepared (0.6% w/v) TTC solution. The test tubes were incubated at room temperature for 18 hour in dark. After 18 hour the content of test tube turns red. The TTC solution was drained off from the test tubes and the remaining leaf tissues in test tubes was gently washed with distilled water 2-3 times to completely remove TTC solution. The red color of leaf tissue was extracted by adding 5 ml of absolute alcohol in each test tube followed by boiling the content on water bath for 20 minutes. The extinction value was read at 530 nm and expressed in terms of dry weight equivalent. Dry weight equivalents of each sample were determined by keeping 50 mg of fresh leaves in an oven at 80 °C for 24 hour. the cellular respiratory ability was expressed as a percent with respect to control.

4.3.3 Carbohydrate estimation (Loewus, 1952)

Reagents: a) Anthrone reagent: Dissolve 200 mg anthrone in 100 ml of ice cold 95% H_2SO_4 (Sulphuric acid). Freshly prepared solution was used.

Procedure:

In a test tube, 200 µl of the plant extract were taken and volume was made up to 1 ml and Blank was prepared using 1 ml distilled water and then 4 ml of anthrone reagent was added to each test tube. Test tubes were heated for eight minutes in a boiling water bath. Cooled rapidly and read the green to dark green colour at 630 nm.

4.3.4 Protein estimation (Lowry *et al.,* 1951)

Reagents: a) Reagent A: 2% sodium carbonate in 0.1 N sodium hydroxide.

b) Reagent B: 0.5% CuSO4.5H2O in 1% sodium potassium tartarate.

c) Reagent C: 50 ml of reagent A was mixed with 1 ml of reagent B just before use.

d) Reagent D: Folin Ciocalteau's phenol reagent was diluted with water in 1:1 before use.

Procedure:

To 0.5 ml of the approximately diluted samples of protein in a test tube, 2.5 ml of reagent C was added. The contents were mixed well and allowed to stand for 10 min at room temperature. Then 0.25 ml of reagent D was added and mixed rapidly. After 30 minutes, the intensity of blue colour was read at 520 nm. The amount of protein in samples was calculated from standard curve prepared by taking different concentrations of bovine serum albumin (20-100 µg).

4.3.5 α-amylase activity (Muentz, 1977)

Reagents: a) Substrate solution: (150 mg soluble starch, 600 mg potassium dihydrogen phosphate and 20 mg of anhydrous calcium chloride in 100 ml of distilled water, boiled for 1 minute, cooled and filtered).

b) 0.1 M EDTA.

c) Iodine solution: (25.4 mg iodine dissolved with 0.4 g potassium iodide in 100 ml distilled water).

Procedure:

α-amylase specific activity was measured in terms of starch used. To 0.5 ml of enzyme extract added 1 ml of substrate solution. The mixture was incubated for half an hour followed by addition of 0.1 ml of 0.1 M EDTA. To 0.2 ml of reaction mixture added 3 ml of iodine solution. The concentration of left over starch was measured spectrophotometrically at 630 nm using starch (50 µg/ml) as standard. A parallel blank was prepared by addition of water in place of enzyme extract. The enzyme activity was expressed in terms of µg $min^{-1}mg^{-1}$ protein.

4.3.6 Protease Assay (Basha and Beevers, 1975)

Reagents: a) casein solution (1% in 0.1 M phosphate buffer, pH 6.0).

b) TCA solution (15%, w/v).

c) Reagents used for Lowry *et al.* (1951).

Procedure:

To 0.5 ml of enzyme extract was added 0.5 ml of casein solution (1% in 0.1 M phosphate buffer, pH 6.0) and the mixture incubated at 37 C for 1 hour to precipitate the proteins, 1 ml of TCA solution (15%, w/v) was added to the above mixture and the contents were centrifuged at 10,000 rpm for 5 minutes to get the amino acids released in supernatant. Further, this supernatant was used for estimation of enzyme activity by method of Lowry *et al*. (1951). Specific activity was calculated against 50 µg/ml tyrosine as standard and expressed as g h^{-1} mg^{-1} protein.

4.3.7 Peroxidase Assay (Shannon *et al.,* 1966)

Reagents: a) 0.05 M guaiacol in 0.1 M phosphate buffer (pH 6.5).

b) 0.8 M H_2O_2 (Hydrogen peroxide).

Procedure:

The reaction mixture contained 3 ml of 0.05 M guaiacol in 0.1 M phosphate buffer(pH 6.5), 0.1 ml of enzyme extract and 0.1 ml of 0.8 M H_2O_2. The reaction mixture without H_2O_2 was measured as a blank. The reaction was initiated by adding H_2O_2 and rate of change in absorbance was recorded at 470 nm for 3 minutes at an interval of 30 seconds. Tetrahydroguaiacol is absorbed at 470 nm. Peroxidase activity has been defined as change in absorbance/min/g of tissue.

4.3.8 Superoxide dismutase assay (Marklund and Marklund, 1974)

Reagents: a) 0.1 M Tris HCl buffer (pH8.2).

b) 6 mM EDTA.

c) 6 mM Pyrogallol solution.

Procedure:

To a cuvette, 1.5 ml of 0.1 M Tris HCl buffer (pH 8.2), 0.5 ml of 6 mM EDTA, 1 ml of 6 mM pyrogallol solution and 0.1 ml of enzyme extract was added. Absorbance was recorded at 420 nm after an interval of 30 seconds up to 3 minutes. A unit of enzyme activity has been defined as the amount of enzyme causing 50% inhibition of auto-oxidation of pyrogallol observed in blank.

4.4 Statistical analysis

All the experiments were performed in a completely randomized block design and performed twice. For each treatment five replications were maintained. The data collected from study was subjected to one way ANOVA with Tukey's test in SPSS 18.

CHAPTER-5

RESULTS AND DISCUSSION

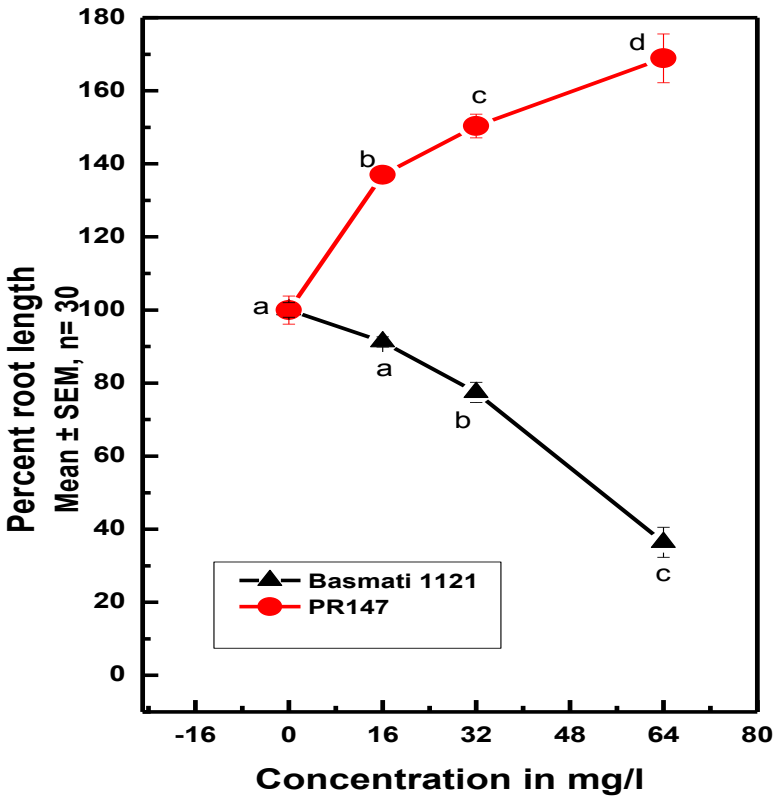
5.1General results and references

During seedling growth it was observed that in the control group of both the rice varieties the germination of seedlings started within 48 hours and in fluoride concentrations the germination started after 48 hours. After 7 days of seedling germination it was observed that only 90% seeds germinate of the total seeds kept for germination.

5.2 Effect of NaF on seedling growth

5.2.1 Effect of NaF on root length

Fig-1: Effect of different concentrations of NaF on root length of two varieties of rice.



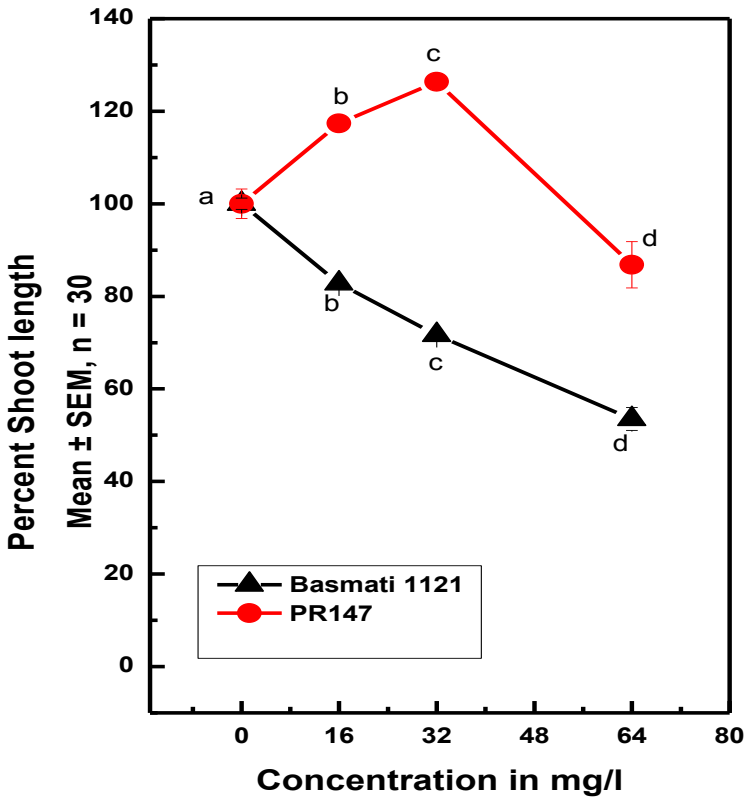
Different alphabets along each line represents significant differences over control at P ≤ 0.05 applying Tukey's test.

When the two varieties of rice were subjected to different concentration of NaF, reduction was observed in root length of Basmati 1121 variety while the PR147 variety showed increase in root length. In Basmati 1121, at 16 mg/l concentration, reduction of 9% was observed. Same trend followed at higher concentrations. At highest concentration of 64 mg/l, a large reduction of nearly 64% was observed. This decrease in root length was significant compared to control.

However, in PR147 variety enhancement in the growth was observed. The increase was 40% at 16 mg/l which increased to nearly 70% at 64 mg/l concentration. The increase was significant at all concentration with control.

5.2.2 Effect of NaF on shoot length

Fig-2: Effect of different concentrations of NaF on shoot length of two varieties of rice.



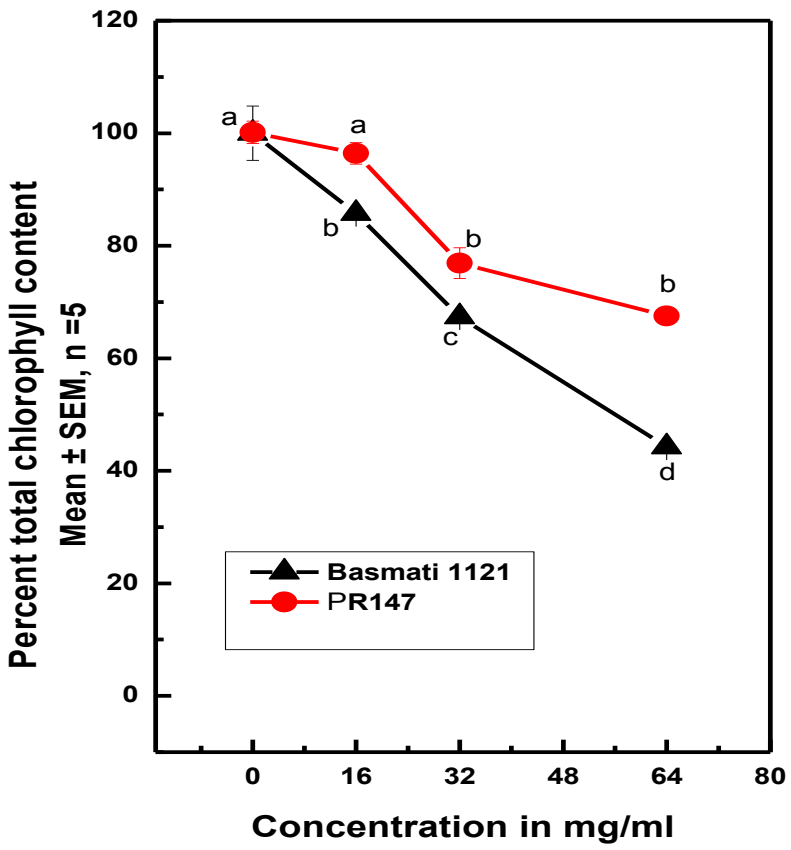
Different alphabets along each line represents significant differences over control at P ≤ 0.05 applying Tukey's test.

Like root length, similar trend was followed in shoot length up to treatment with 32 mg/l concentration of fluoride. In Basmati 1121 approximately 20% reduction was observed on treatment with 16 mg/l sodium fluoride which reached to reduction of nearly 50%. However, in PR147 variety, initially the shoot length increased to nearly 18% with 16 mg/l concentration and 32% with 32 mg/l concentration. After further increase in concentration, a sharp decrease in shoot length was observed. The percent decrease in shoot length at 64 mg/l was around 20% compared to control.

5.3 Effect of NaF on physiological parameters of two varieties of rice

5.3.1 Effect on total chlorophyll content

Fig-3: Effect of different concentrations of NaF on total chlorophyll content of two varieties of rice.



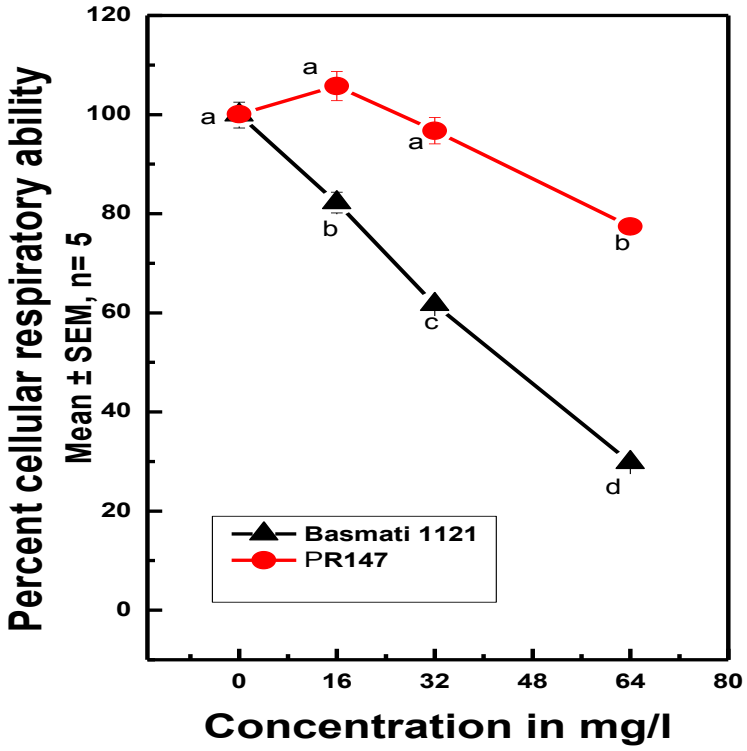
Different alphabets along each line represents significant differences over control at $P \leq 0.05$ applying Tukey's test.

Chlorophyll content of both the varieties was reduced as the amount of fluoride concentration was increased from control to 64 mg/l. In Basmati 1121 variety, a constant significant reduction of nearly 18%, 36% and 62% was observed on treatment with 16, 32 and 64 mg/l concentration, respectively.

However in PR147 variety, at initial concentration of 16 mg/l, insignificant decrease of nearly 3% was observed. Further, at higher concentrations of 32 mg/l and 64 mg/l, significant decrease of nearly 22% and 30% was observed.

5.3.2 Effect on percent cellular respiratory ability

Fig-4: Effect of different concentrations of sodium fluoride on percent cellular respiratory ability of two varieties of rice.



Different alphabets along each line represents significant differences over control at P ≤ 0.05 applying Tukey's test.

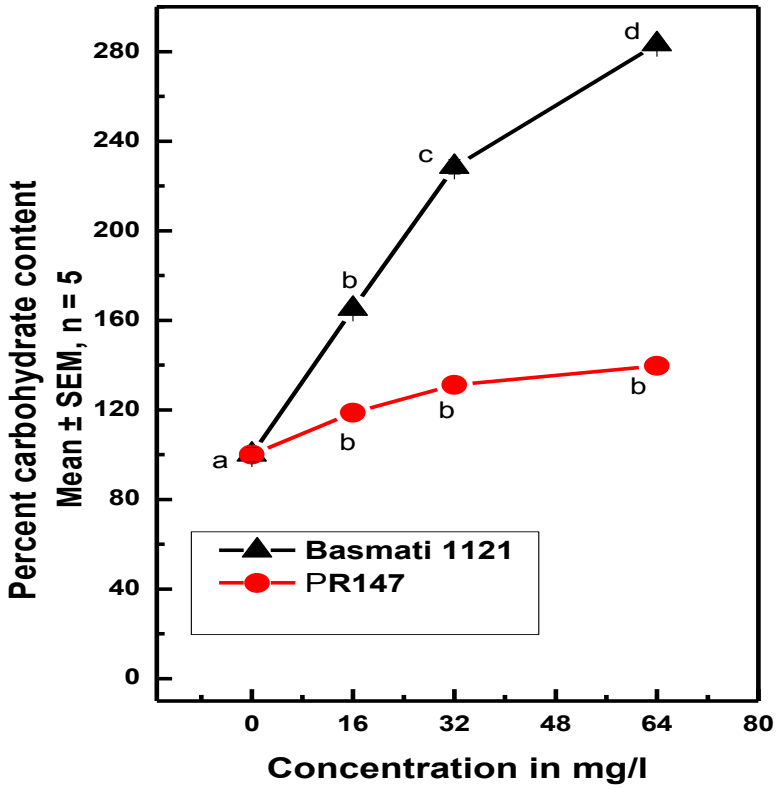
Percent cellular respiratory ability of both the varieties was reduced as the amount of fluoride concentration was increased from control to 64 mg/l. In Basmati 1121 variety, a constant significant reduction of nearly 20%, 40% and 70% was observed on treatment with 16, 32 and 64 mg/l of fluoride concentration,

respectively. However in PR147 variety, at initial concentration of 16 mg/l, the total cellular respiratory ability was increased by 5%. Further, at higher concentrations of 32 mg/l and 64 mg/l, significant decrease of nearly 10% and 30% was observed.

5.4 Effect of NaF on macromolecules and their hydrolyzing enzymes

5.4.1 Effect on water soluble carbohydrate

Fig-5: Effect of different concentrations of NaF on total carbohydrate content of two varieties of rice.



Different alphabets along each line represents significant differences over control at P ≤ 0.05 applying Tukey's test.

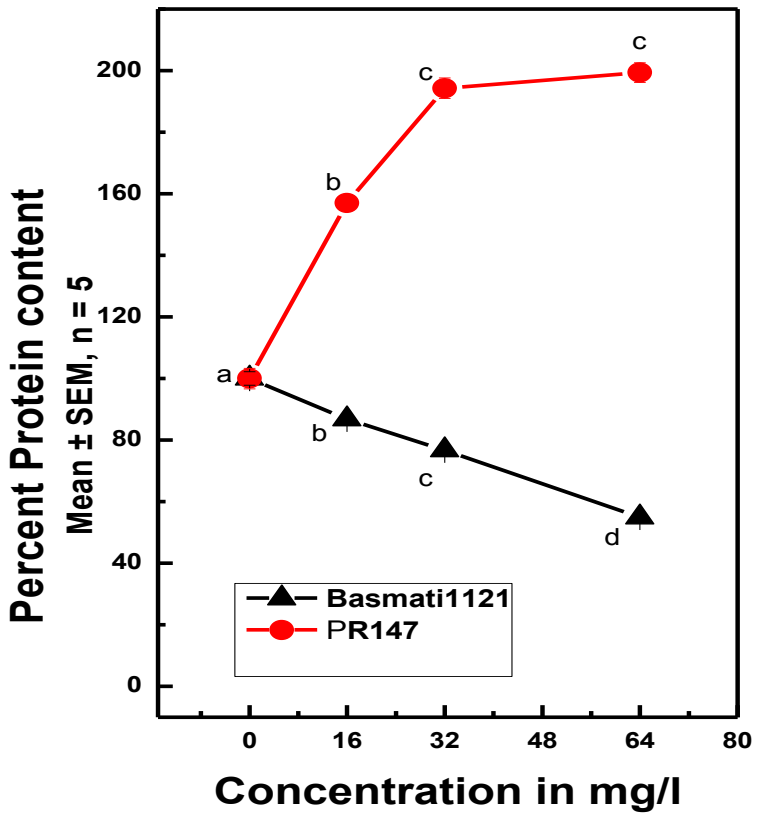
The carbohydrate content of both varieties increased with increase in concentration of sodium fluoride. However, the increase varied in two varieties to a greater extent. In Basmati 1121 , a significant increase of nearly 1.65, 2.3 and 2.9

times in water soluble carbohydrate content were observed with 16, 32 and 64 mg/l concentration of NaF concentration.

However in PR147, initially significant increase of nearly 1.2 times was observed with 16 mg/l. Further, at 32 mg/l, a small increase of 1.3 times was observed which further increased insignificantly to 1.35 times with 64mg/l concentration.

5.4.2 Effect on water soluble protein

Fig-6: Effect of different concentrations of NaF on total protein content of two varieties of rice.



Different alphabets along each line represents significant differences over control at P ≤ 0.05 applying Tukey's test.

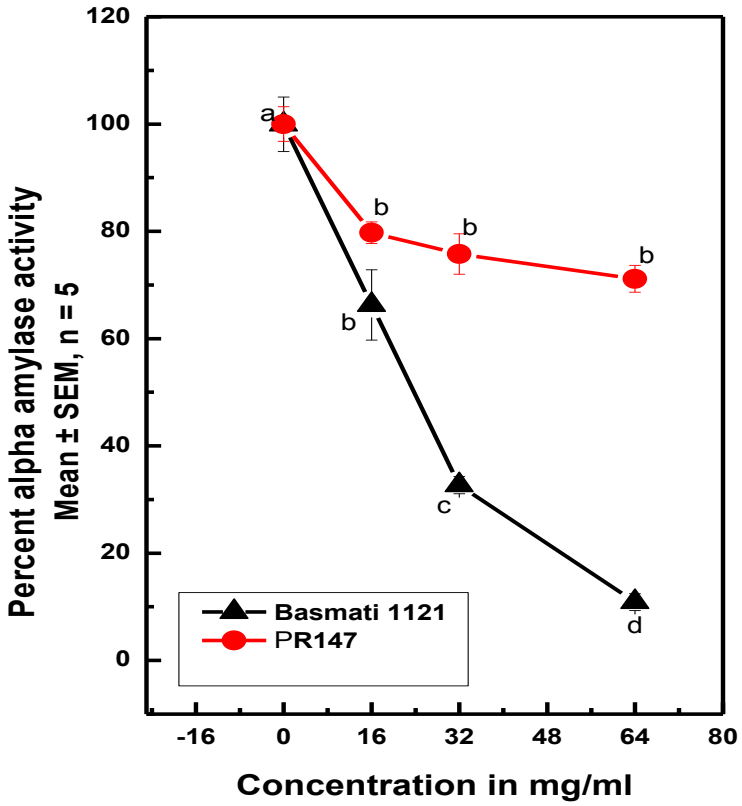
The protein content of the two varieties also affected variably with different concentrations of NaF. In PR147, an increase in protein content was observed while decrease in protein content was observed in Basmati 1121 variety. In PR147, the protein content increased nearly 1.6 times at 16 mg/l concentration

and 1.9 times at 32 mg/l concentration. However, at 64 mg/l concentration, only a small insignificant increase was observed as compare to protein content at 32 mg/l concentration.

In Basmati 1121, the protein content decreased with increase in concentration of sodium fluoride. The protein content decreased to approximately 18%, 30% and 55% with increase in concentration of sodium fluoride from 16 to 64 mg/l. This decrease was observed to be significant at all concentrations compared to control.

5.4.3 Effect on α-amylase activity

Fig-7: Effect of different concentrations of NaF on α-amylase activity of two varieties of rice.



Different alphabets along each line represents significant differences over control at P ≤ 0.05 applying Tukey's test.

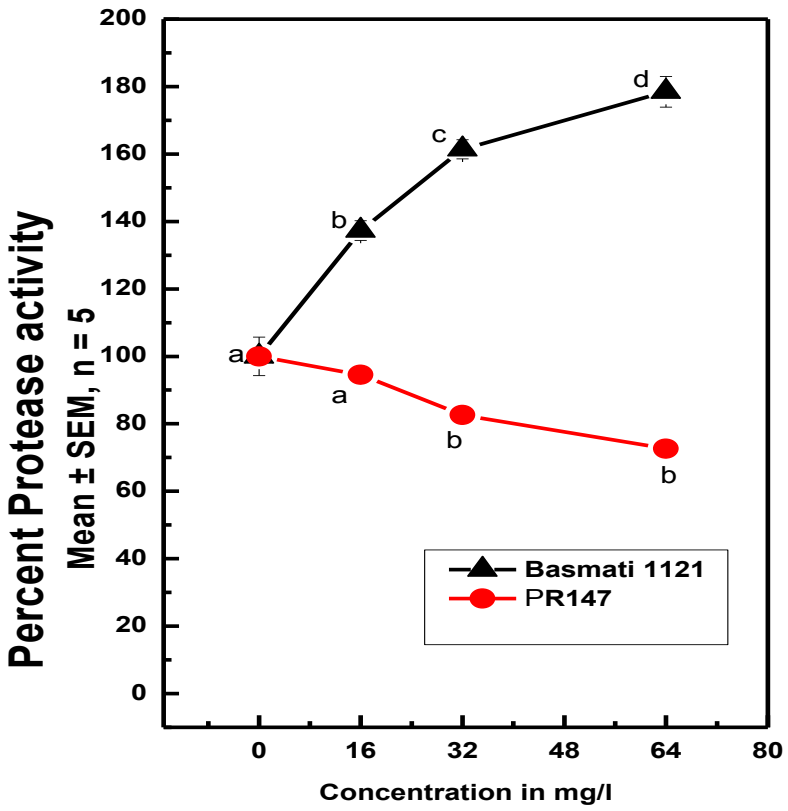
The carbohydrate content was increased with increase in fluoride concentration as the activity of hydrolyzing enzyme was decreased. Reduction in α-amylase activity

was observed in both the varieties of Basmati 1121 and PR147. In Basmati 1121 35%, 70% and 90% decrease was observed, respectively at 16 mg/l, 32 mg/l, and 64 mg/l of fluoride concentration.

Further in PR147 variety there is a decrease of 22%, 25%, 30% was observed as the concentration of fluoride increases from 16 mg/l to 64 mg/l. The decrease in enzyme activity was more in Basmati 1121 due to fluoride stress.

5.4.4 Effect on protease activity

Fig-8: Effect of different concentrations of NaF on protease activity of two varieties of rice.



Different alphabets along each line represents significant differences over control at P ≤ 0.05 applying Tukey's test.

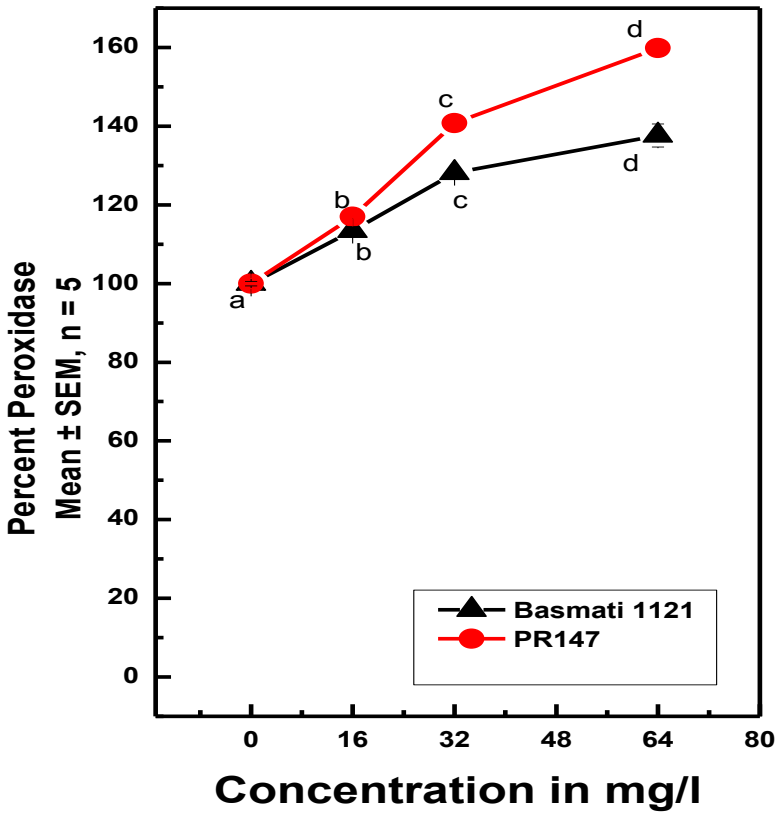
Like protein content, the protease activity also varied in both the varieties. In Basmati 1121 , increase in protease activity was observed with increase in fluoride concentration and at highest concentration (64 mg/l) of fluoride, the protease

activity increased by 1.78 times, while in case of PR147 variety the protease activity decreased with increase in fluoride concentration. At lower concentration of 16 mg/l fluoride, an insignificant decrease of nearly 7% was observed. However, at 32 and 64 mg/l fluoride concentrations, significant decrease of 23% and 35% in protease activity was observed, respectively.

5.5 Effect of oxidative stress caused by NaF

5.5.1 Effect on Peroxidase activity

Fig-9: Effect of different concentrations of NaF on peroxidase activity of two varieties of rice



Different alphabets along each line represents significant differences over control at P ≤ 0.05 applying Tukey's test.

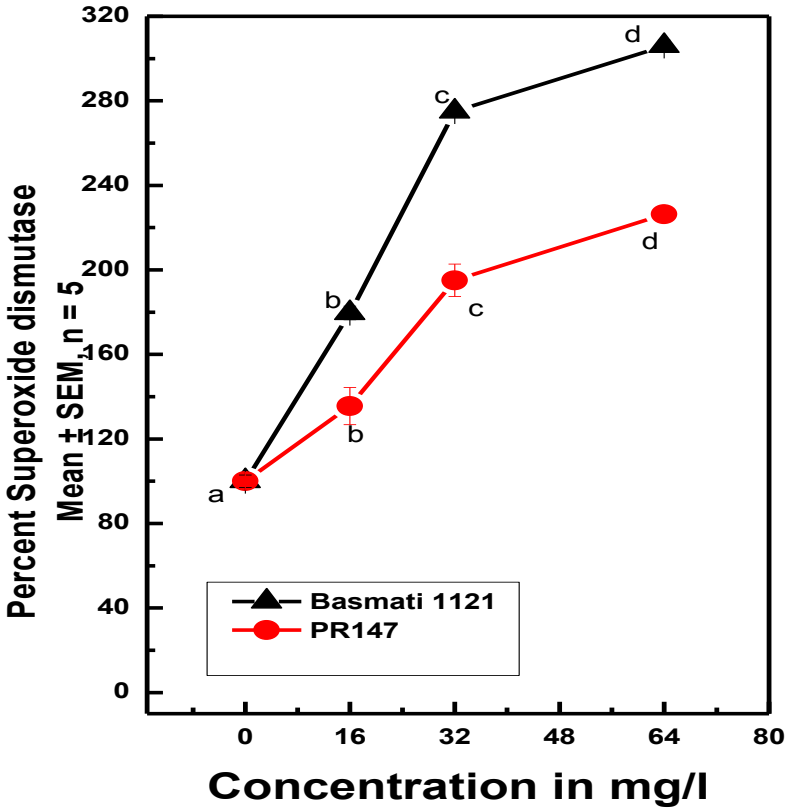
Peroxidase activity increased in both the rice varieties with increase in fluoride concentration. However, the increase was more in PR147 variety compared to Basmati 1121. In PR147, the increase in peroxidase activity was nearly 1.15, 1.4,

1.6 times at 16, 32 and 64 mg/l concentration of sodium fluoride. This increase was observed significant compared to control.

Similarly in Basmati 1121, increase in activity of peroxidase enzyme at 16, 32 and 64 mg/l concentration of fluoride was nearly 1.13, 1.25 and 1.38 times and this increase was observed significant compared to control.

5.5.2 Effect on superoxide dismutase activity

Fig-10: Effect of different concentrations of NaF on superoxide dismutase activity of two varieties of rice



Different alphabets along each line represents significant differences over control at P ≤ 0.05 applying Tukey's test.

Superoxide dismutase activity increased in both the rice varieties with increase in fluoride concentration. However, Basmati 1121 variety compared to PR147 has high SOD activity. In Basmati 1121, increase in activity of SOD enzyme at 16, 32

and 64 mg/l concentration of fluoride was nearly 1.8, 2.75 and 3.1 times and this increase was observed significant compared to control.

Similarly in PR147, the increase in SOD activity was nearly 1.3, 1.7, 2.25 times at 16, 32 and 64 mg/l concentration of sodium fluoride. This increase was observed significant compared to control.

5.6 Discussion

The two rice varieties showed variation in seedling growth on treatment with different concentrations of sodium fluoride. The root and shoot length of Basmati 1121 decreased with increase in fluoride concentration while in PR147, the seedling length initially increased till 32 mg/l concentration of sodium fluoride, then sharply decreased in shoot length. The decrease in root and shoot length in response to fluoride has been reported by Gupta *et al.,* (2009) and Sabal *et al.,* (2006).

Further, enhancement in seedling length in response to fluoride concentration has also been reported Pant *et al.,* (2008) while working on tomato. The possible reason may be that PR147 may be more tolerant to fluoride due to internal/genetic factors. However, when the concentration increases a limit, it also shows reduction in seedling length.

In our study, the chlorophyll content of both the varieties decreased with increase in sodium fluoride concentration and this effect was more in Basmati 1121. The results are in agreement of earlier reports of number of researchers (Bhargava and Bhardwaj, 2010; Gupta *et al.,* 2009; Kumar and Rao, 2008). Wallis *et al.,* 1974 reported that the biochemical basis of this may be incorporation of gamma-aminolevunilic acid into chlorophyll synthetic pathway.

Further, Chang and Thompson, (1966) reported chloroplasts as the site of fluoride accumulation and disruption of chloroplast membrane of seedlings grown under high fluoride concentrations. Further, the difference in response to fluoride concentration by two rice varieties may be attributed to differences in fluoride tolerance in two varieties. Similar observation has also made by Kumar and Rao, (2008) while working on two cultivars of mulberry.

The total cellular respiratory ability of the two rice varieties also decreased and the results are similar as reported by many investigators (Hill *et al.,* 1959). The

possible reason for this may be the result of inhibition of enolase (Miller, 1958; Warburg and Christian, 1942).

The water soluble carbohydrate content was increased with increase in fluoride concentration in both the varieties and the studies done by Gupta *et al.,* (2009) and Li and Ni, (2009) support the same. The protein content of Basmati 1121 variety was decreased with increase in fluoride concentration and the study done by Li and Ni, 2009 observed the same decrease in protein content of tea leaves while in PR147 variety the protein content was increased. When the macromolecule hydrolyzing enzymes were studied, it was observed that the α-amylase activity decreased in both varieties and protease activity increased in Basmati 1121 while decreased PR147 variety with increase in fluoride concentration. The probable cause of increase in water soluble carbohydrate content may be decrease in activity of its hydrolyzing enzyme amylase. Previously, some researchers have also reported the inhibition of water soluble carbohydrate catabolization in response to fluoride (Gupta *et al.,* 2009; Li and Ni, 2009). Gupta *et al*., (2009) has reported this as a mechanism adopted by rice to reduce the effect of fluoride stress. Further, Sarkar *et al.,* 1982 also reported inhibition in amylase activity in response to fluoride concentration.

Similarly, the results of increase in protein content in PR147 and decrease in Basmati 1121 may be attributed to corresponding activity of protease enzyme. The difference may again be attributed to tolerance power of two varieties.

The stress controlling enzymes plays an important role in growth of seedling. Two antioxidant enzymes viz. peroxidase and superoxide dismutase were studied. The activities of both the enzymes enhanced with increase in fluoride concentration. The enzymes are well known for their production under environmental stress conditions (Kumar *et al.,* 2009; Choudhary and Bohra, 1989). The increase in activity of two enzymes indicates the production of reactive oxygen species under fluoride stress conditions. A study on peroxidase by Kumar *et al.,* (2009) reported an increase in enzyme activity in Mulberry leaves.

In our study we observed an increased in SOD activity and it may be due to exposure of fluoride causing increased biosynthesis of SOD (Wilde and Yu, 1998). Although Wilde and Yu, 1998 observed an enhancement in SOD activity but at

very low fluoride concentrations but at higher concentrations the activity was reduced.

So the present study concludes that in general, the high concentration of fluoride produces stress as indicated by various growth, physiological and biochemical parameters under study. However, the effect differs in different varieties depending on their tolerance power to the fluoride stress.

CONCLUSIONS AND FUTURE PROSPECT

Conclusions

In our present study the effect of natrium fluoride on growth and physiology of *O. sativa* L. was studied taking two varieties of rice and following important conclusions were obtained:

- Reduction in root and shoot length was observed in Basmati 1121 and enhancement was observed in PR147 affecting the total growth of the plant.
- Chlorophyll content and percent cellular respiratory ability was decreased at high fluoride concentrations affecting the photosynthetic ability of the plant.
- The macromolecules and their hydrolyzing enzymes were also affected by the fluoride exposure.
- Oxidative stress caused due to fluoride exposure leads to an increase in antioxidant enzyme activity. Peroxidase and SOD activity was increased.

Table:1 Effect of natrium fluoride on different parameters:

S. No	Variety Parameters	Basmati 1121	PR147
1.	Root length	-ve	+ve
2.	Shoot length	-ve	+ve
3.	Chlorophyll content	-ve*	-ve
4.	Percent cellular respiratory ability	-ve*	-ve
5.	Carbohydrate content	+ve*	+ve
6.	Protein content	-ve	+ve
7.	α-amylase activity	-ve*	-ve
8.	Protease activity	+ve	-ve
9.	Peroxidase activity	+ve*	+ve
10.	Superoxide dismutase activity	+ve*	+ve

+ve= Positive, -ve= Negative, * shows highly affected

The Basmati 1121 variety was more affected by the fluoride exposure as compared to PR147. Although in PR147 an enhancement in root and shoot length was observed and this can be due to various alterations in its metabolic activity.

Future Prospect

- Further studies can be carried out to identify the reasons responsible for enhancement in growth of PR147 on treatment of NaF.
- Effect of Fluoride present in ground water and the concentration of Fluoride accumulated in the plant.
- Transfer of total amount of fluoride from one trophic level to another.

SUMMARY

Many studies indicate that high concentration of fluoride negatively affects the physiological and biochemical activities of the plants and hence, reduces the plant growth and yield. So a study was required to know how fluoride was affecting the crop plants especially those which were used as a source of food.

In our present study the effect of natrium fluoride on growth and physiology of two rice varieties were studied. It was found that there is a significant effect on root and shoot length of the seedling with increase in concentration of fluoride. In Basmati 1121, seedling growth was decreased with an increase in fluoride concentration. While in PR147 there is an enhancement of growth in both root and shoot length except at 64 mg/l where the growth of shoot was sharply decreased.
In Basmati 1121, at 16 mg/l concentration, reduction of 9% in root length was observed which increased to 64% at 64 mg/l concentration of sodium fluoride.

However, in PR147 variety enhancement in root length was observed, this increase was 1.40 times at 16 mg/l and nearly 1.70 times at 64 mg/l concentration.

Like root length, similar trend was followed in shoot length up to treatment with 32 mg/l concentration. In Basmati 1121 approximately 20% reduction was observed on treatment with 16 mg/l sodium fluoride which reached to reduction of nearly 50% at 64 mg/l sodium fluoride. However, in PR147 variety, initially the shoot length increased to nearly 18% with 16 mg/l concentration and 32% with 32 mg/l concentration of sodium fluoride. After further increase in concentration, a sharp decrease in shoot length was observed.

The physiological parameters i.e. chlorophyll and respiration were studied. It was observed that the chlorophyll content was decreased in both varieties of rice with increase in fluoride concentration from 16 mg/l to 64 mg/l. In PR147, initially at 16 mg/l concentration, the decrease in chlorophyll content was not significant but at higher concentration it decreased significantly. At highest concentration of 64 mg/l, the percent reduction in chlorophyll content was nearly 30% and 62% in PR147 and Basmati 1121, respectively.

Percent cellular respiratory ability of both the varieties reduced on treatment with different concentrations of sodium fluoride. In Basmati 1121, initially at 16 mg/l concentration, the percent reduction in cellular ability was nearly 20% which

further increased to nearly 75% at 64 mg/l. However, in PR147 variety, initially there was slight increase in percent respiratory ability at 16 mg/l concentration, but at 64 mg/l concentration of sodium fluoride, a significant reduction of nearly 22% was observed.

The carbohydrate content was increased significantly while α-amylase activity decreases significantly in both rice varieties with increase in fluoride concentration. However, the effect varied in two varieties to greater extent. In Basmati 1121, a significant increase of nearly 1.65, 2.3 and 2.9 times and in PR147, 1.2, 1.3 and 1.35 times in water soluble carbohydrate content were observed with 16, 32 and 64 mg/l concentration of sodium concentration.

α-amylase activity was decreased in both the varieties of Basmati 1121 and PR147. In Basmati 1121 35%, 70% and 90% decrease was observed, respectively at 16 mg/l, 32 mg/l, 64 mg/l. Further in PR147 there is a decrease of 22%, 25%, 30% was observed as the concentration of fluoride increases. The decrease in enzyme activity was more in Basmati 1121 due to fluoride stress.

Further, in Basmati 1121 the protein concentration decreased significantly and the protease concentration increased but in PR147 the protein concentration increases with fluoride concentration and the protease concentration decreases.

At 64 mg/l fluoride concentration, the increase in protein content in PR147 was nearly 1.9 times while protease activity decreased to nearly 35%. However, reverse was observed in Basmati 1121 where the protein content decreased to 55% while protease activity increased by 1.78 times on treatment with 64 mg/l concentration.

Further, the effect of fluoride on oxidative stress enzymes was also studied. The activity of peroxidase and SOD increased with increase in fluoride concentration indicating the production of stress in response to different concentrations of sodium fluoride. The increase in activities of the enzymes was observed in both rice varieties. Peroxidase activity increased to nearly 1.15, 1.4, 1.6 times in PR147 and 1.13, 1.25 and 1.38 times in Basmati 1121 in response to 16, 32 and 64 mg/l concentration of sodium fluoride. This increase was observed significant compared to control.

Superoxide dismutase activity increased in both the rice varieties with increase in fluoride concentration. In Basmati 1121, increase in activity of peroxidase enzyme at 16, 32 and 64 mg/l concentration of fluoride was nearly 1.8, 2.75 and 3.1 times.

Similarly in PR147, the increase in peroxidase activity was nearly 1.3, 1.7, 2.25 times at 16, 32 and 64 mg/l concentration of sodium fluoride. This increase in activity of SOD was observed significant compared to control.

So the present study concludes that in general, the high concentration of fluoride produces stress as indicated by various growth, physiological and biochemical parameters under study. However, the effect differs in different varieties depending on their tolerance power to the fluoride stress.

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