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Full Length Research Article

Antioxidant Enzymes Activity and Biochemical Contents in Paddy (*Oryza sativa* var. Deluxe Ponni) Under Fluoride Stress

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Abstract

Fluoride ion is toxic and harmful to living organisms. The present study investigated the impact of different fluoride ion concentrations (0, 1, 2, 5, 10, 50, and 100 ppm) on some antioxidant enzyme activities (SOD, CAT, and APX) and biochemical contents (protein and carbohydrate) levels in paddy (*Oryza sativa*, var. Deluxe Ponni) for a period of 15 days. The obtained results were showed that the antioxidant enzyme activity increased and the biochemical contents decreased with the increase in fluoride ion concentration compared to the control.

Key words: *Oryza sativa*, Fluoride, Antioxidant, Enzyme, Catalase, Superoxide dismutase

Fluoride occurs naturally in soil, water, and atmosphere. The main sources of fluoride in the environment are by weathering of rocks, industrial emissions, atmospheric deposition, etc. [1]. High consumption of fluoride by animals leads to fluorosis. WHO recommended the maximum permissible limit of fluoride in drinking water be 1.5 mg/L [2]. When fluoride exposure surpasses the allowed limits, it has an impact on people, animals, and plants [3]. The bones and teeth are affected by fluoride buildup in the human body. Elevated fluoride levels in the growing enamel are the root cause of dental fluorosis [4]. Based on the atmospheric concentration needed to harm plants, fluoride is the most phototoxic known air contaminant. Exposure to fluoride for a prolonged period primarily leads to chlorosis and then necrosis in the leaves of plants. Fluoride has long been known as a potent metabolic inhibitor, interfering with the overall responses of plants including seed germination, protein synthesis gene expression and the overproduction of ROS such as superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), and hydrogen peroxide (H_2O_2) [5-6]. High fluoride ion reduces photosynthesis by reducing chlorophyll, chloroplast degradation, and the Hills reaction [7]. Reactive oxygen species hinder photosynthesis, lipid peroxidation, cell disruption, DNA damage, and ATP generation in response to stress [8]. Plants have evolved antioxidative defense mechanisms to combat ROS under fluoride ion stress. Antioxidant enzymes such as SOD, CAT, and APX are activated to protect cells from damage [9]. Activation of the antioxidant enzyme is necessary to protect plants from harmful effects.

Many studies have shown the impact of fluoride on germination, physiological and biochemical contents in many plant species, such as *Olive* plants [10], *Oryza sativa* L. [11-12] and *Tritium Aestivum* L [13]. In the present study, the impact of fluoride ion on antioxidant enzyme activities and biochemical contents (carbohydrates and proteins) levels in paddy (*Oryza sativa*, var. Deluxe Ponni) were investigated.

MATERIALS AND METHODS

Collection of seed

Paddy (*Oryza sativa*, var. Deluxe Ponni) seeds were collected from the agriculture department, Tirunelveli. Selected seeds of uniform size were surface sterilized with 0.1% mercuric chloride for three minutes and were washed thoroughly with distilled water and blotted.

Preparation of stock solution

The fluoride stock solution (1000 ppm) was prepared by dissolving 2.21 g of anhydrous sodium fluoride in 1000 mL of distilled water. Further, six different concentrations of fluoride (1, 2, 5, 10, 50, and 100 ppm) were prepared from stock solutions by dilution.

Germination studies

The treated 25 seeds were placed in individual containers labeled as control and sodium fluoride, ranging from 1 to 100 ppm. Each pot was watered with the corresponding fluoride

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solution [14]. After 15 days, the plants were harvested and subjected to the determinations of antioxidant enzyme activity and biochemical contents.

Estimation of antioxidant enzyme activity and biochemical contents

About 1 g of fresh leaf tissues was ground in 10 mL of chilled 50 mM phosphate buffer solution in a prechilled mortar and pestle and centrifuged the homogenate at 11, 180 x g for 10 min at 4 °C. The supernatant liquid was collected and used for the analysis of enzyme activities. SOD activity was determined according to the NBT reduction method [15]. CAT and APX activity was measured by the chemical method [16-17]. The standard phenol-sulphuric acid method [18] was used for the estimation of carbohydrate. Protein was estimated using the Lowery method [19].

RESULTS AND DISCUSSION

The observations made during the study have been discussed in the following. The results are given in the (Table 1-2, Fig 1-5).

Antioxidant enzyme activities

Superoxide dismutase (SOD)

Superoxide dismutase is the most effective intracellular enzymatic antioxidant, which has been proposed to be important in plant stress tolerance and provide the primary line of defense against the toxic effects of elevated levels of ROS. In the present work, superoxide dismutase activity was measured after the 15th day of germination. Table 1 showed that the effect of increasing fluoride ion concentrations with SOD, CAT and APX activities. From the Figure 1, it is clear that the superoxide dismutase enzyme activity is increased linearly with increasing fluoride ion concentrations as compared to the control. Maximum superoxide dismutase activity (0.3905 U/mg) was observed at 100 ppm fluoride stress in accordance with control (0.3237 U/mg). The results are in accordance with the previous studies [9], [20].

Catalase activity (CAT)

Catalase is a heme-containing enzyme that catalyses the dismutation of hydrogen peroxide into water and oxygen. Catalase is involved in the main defense mechanism against the accumulation and toxicity of ROS. Catalase activity on the germination was measured on the 15th day of sowing. Though the production of ROS under fluoride stress is increased at the same time higher concentrations of fluoride ion also increases the CAT activity. Figure 2 showed that CAT activity is increased with increase in fluoride ion concentrations as compared to control. A maximum CAT activity (0.1741 U/mg) at 100 ppm fluoride stress was measured in accordance with control (0.1292 U/mg) (Table 1). The results are corresponded well with the previous reports showing that the oxidative stress causes an elevation of CAT activity, which depends on the duration, type, and intensity of stress [21-22].

Table 1 SOD, CAT, and APX activity in paddy *Oryza sativa* (var. Deluxe Ponni) at different fluoride ion concentration

F – Concentration (ppm)	SOD activity (U/mg Protein)	CAT activity (U/mg)	APX activity (U/mg)
Control	0.3237	0.1292	1.9375
1	0.3276	0.1330	0.8621
2	0.3520	0.1368	1.088
5	0.3581	0.1445	1.4096
10	0.3774	0.1484	2.4689
50	0.3811	0.1671	2.7307
100	0.3905	0.1741	2.8010

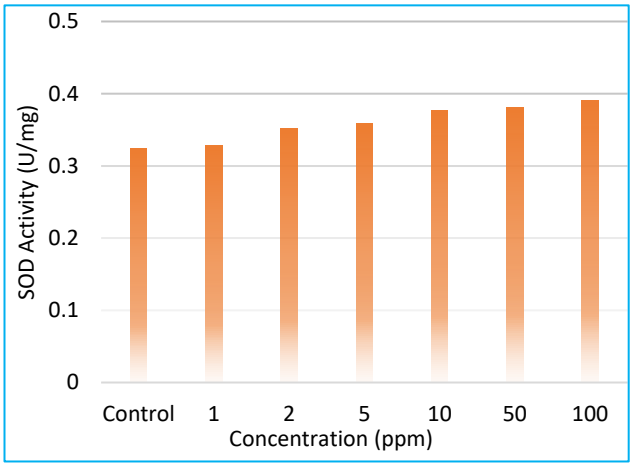

Fig 1 Effect of fluoride ion on SOD activity

Ascorbate peroxidase (APX)

Ascorbate peroxidase is a central component of AsA – GSH cycle, and plays an essential role in the control of intracellular ROS levels. In the present investigation, APX has a higher affinity for the reduction of H_2O_2 to water than CAT. APX activity was measured after the 15th day of germination. The APX activity might be employed as an intrinsic and defensive tool responsible for H_2O_2 degradation under high concentrations of fluoride stress. Figure 3 showed that the level of ascorbate peroxidase enzymes was increased with the increase in fluoride ion concentrations as compared to the control [9]. A maximum APX activity (2.8010 U/mg) was observed at 100 ppm fluoride stress in accordance with control (1.9375 U/mg) (Table 1). The present results are agreed with other reports showing the positive effects of fluoride ion treatment on APX activity in plants [23].

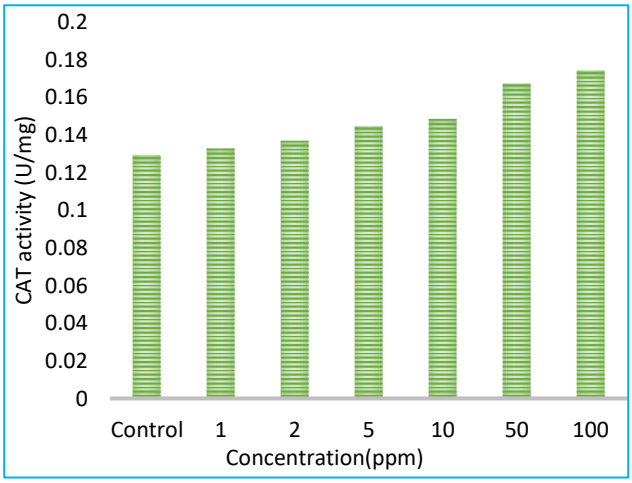

Fig 2 Effect of fluoride ion on CAT activity

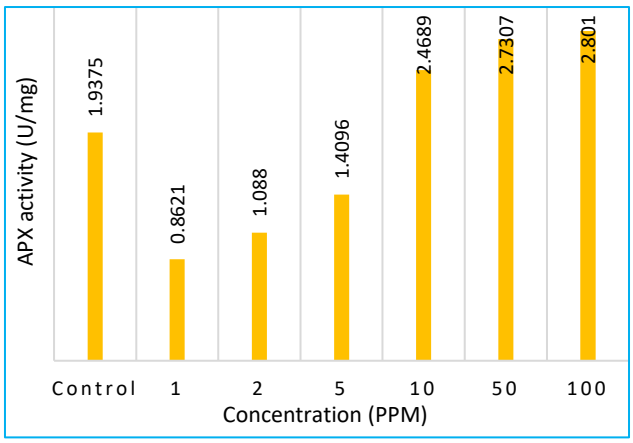

Fig 3 Effect of fluoride ion on APX activity

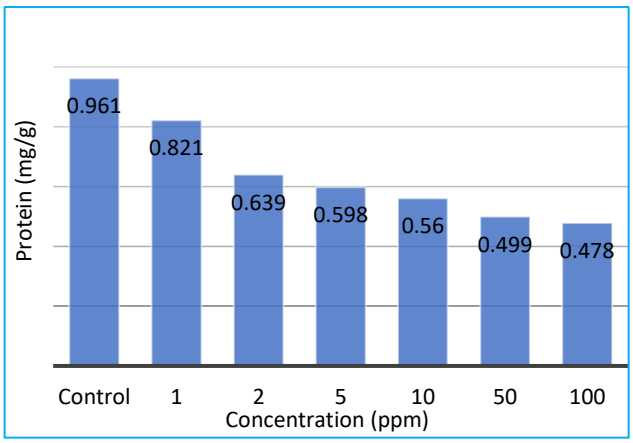

Fig 4 Effect of fluoride ion on protein content

Protein

Proteins, being one of the important organic nitrogenous constituents of plants, play a great role in the compensatory metabolism of a plant species. Higher fluoride ability reduces the protein synthesis which attributed to the modification of free nucleotide and RNA ratios or a decrease in the RNA biosynthesis [25]. In the present study, there was a decrease in total protein content of *Oryza sativa*, (var. Deluxe Ponni) at high fluoride ion concentration as compared to the control (Fig 4) [24]. (Table 2) denotes the minimum protein content (0.478 mg/g) at 100 ppm fluoride stress in accordance with control (0.961 mg/g).

Table 2 Effect of biochemical contents in paddy *Oryza sativa* (var. Deluxe Ponni) at different fluoride ion concentration

Concentration (ppm)	Protein (mg/g)	Carbohydrate (mg/g)
Control	0.961	4.012
1	0.821	4.000
2	0.639	3.832
5	0.598	3.512
10	0.560	3.309
50	0.499	2.397
100	0.478	2.101

Carbohydrate

Generally, fluoride accumulation decreases the plant carbohydrate content which in turn decreases the conversion of carbohydrate into sugar. With the increase in fluoride ion concentration, the concentration of reducing sugars such as glucose, fructose and mannose decreases [26]. The amount of carbohydrate metabolism showed a decreasing trend with increasing fluoride ion concentrations under fluoride stress. (Table 2) clearly revealed that carbohydrate content was significantly lower than controls at all concentrations, a minimum of (3.101 mg/g) carbohydrate content was recorded at the high concentration of fluoride (100 ppm) and in accordance with control (4.012 mg/g).

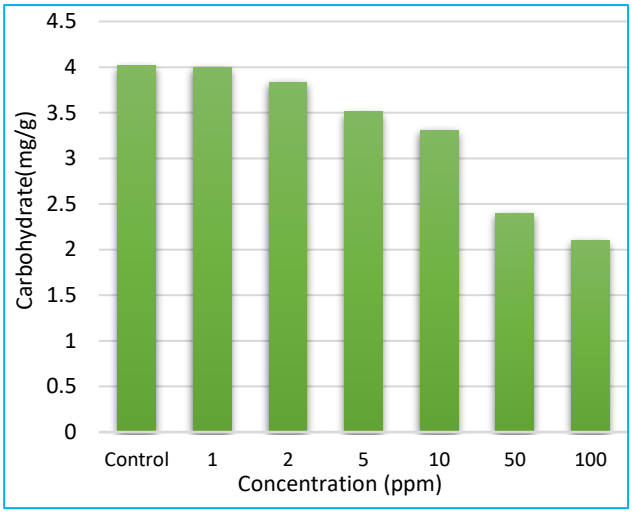

Fig 5 Effect of fluoride ion on carbohydrate content

CONCLUSION

In the present study, it was observed that fluoride stress accelerated the generation of reactive oxygen species (ROS) and stimulated the enhanced activities of antioxidant enzymes (SOD, CAT, and APX) in the plant to counteract the enhanced oxidative stress. It also revealed a significant reduction in protein and carbohydrate content due to oxidative stress induced by fluoride ion in *Oryza sativa* (var. Deluxe Ponni).

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