Ecotoxicology and Environmental Safety 144 (2017) 36–44

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Effect of fluoride on photosynthesis, growth and accumulation of four widely cultivated rice (*Oryza sativa* L.) varieties in India

Naba Kumar Mondal

Environmental Chemistry Laboratory, Department of Environmental Science, The University of Burdwan, 713104, India

ARTICLE INFO

Keywords:
Fluoride contamination
Rice
Germination
Accumulation
Biochemical
Enzyme
Biomass

ABSTRACT

Long-term use of fluoride contaminated groundwater to irrigate crops; especially paddy rice (*Oryza sativa* L.) has resulted in elevated soil fluoride levels in Eastern India. There is, therefore, growing concern regarding accumulation of fluoride in rice grown on these soils. A laboratory experiment was conducted to investigate the effect of F on germination and phytotoxicity of four varieties of rice (*Orzya sativa* L.) (MTU-1010; IET-4094; IET-4786 and GB-1) grown in petri dish in a green house with inorganic sodium fluoride (NaF). Three different levels (0, 5, 10 and 20 mg/L) of NaF solution were applied. At the end of the experiment (28 days), biochemical analysis (pigment, sugar, protein, amino acid and phenol), lipid peroxidation, root ion leakage and catalase activity along with fluoride accumulation and fresh and dry weight of roots and shoots of four cultivars were measured. The results revealed that all the four studied varieties exhibited gradual decrease of germination pattern with increasing concentration of F. Pigment and growth morphological study clearly demonstrated that the variety IET-4094 was the least influenced by F compare to the other three varieties of rice. The translocation factor (TF) was recorded to be the highest for variety IET-4786 (0.215 ± 0.03) at 5 mg/L F concentration. All the four varieties showed higher level of fluoride accumulation in root than in shoot. Variable results were recorded for biochemical parameters and lipid peroxidation. Catalase activity and relative conductivity (root ion leakage) gradually increased with increasing F concentration for all the four varieties. It is speculated that fluoride accumulation in rice straw at very high levels will affect the feeding cattle and such contaminated straw could be a direct threat to their health and also, indirectly, to human health via presumably contaminated meat and milk.

1. Introduction

Now-a-days, the whole world is facing an unprecedented ecological contamination problem. One such severe ecological problem is fluoride (F) phytotoxicity (Saini et al., 2012). F is released from a number of industries (Mackowiak et al., 2003) and pollute all sectors of the biosphere (Cai et al., 2016). The major source of fluoride contamination is water which causes fluorosis in the endemic areas. However, certain food items also contribute significantly to the total intake of fluoride (Gulati et al., 1993; Singh et al., 1993). Previous literature (Shitumbanuma et al., 2007; Ayoob and Gupta, 2006) revealed that elevated concentration of fluoride in groundwater is responsible for serious health problem in many parts of the world. A huge population (200 million) of the world including India and China are severely affected by dental, skeletal, and nonskeletal fluorsosis (Yang et al., 2003). The maximum permissible limit of fluoride in drinking water is 1.5 mg/L as recommended by World Health Organization (WHO, 1984). However, there is no stringent threshold limit of F in soil and plants above which the ingestion may be detrimental to human health. Excessive use of fluoride contaminated ground water for irrigation is prevalent in many fluoride endemic areas, which affect the crops considerably (Chakrabarti and Patra, 2013). Pant et al. (2008) reported that fluoride is absorbed by plant roots and then transported via xylemic flow to different parts of the plant where it gets accumulated. The effect of F on germination, physiological and biochemical parameters in different plant species has been studied by many researchers (Chakrabarti and Patra, 2013; Datta et al., 2012; Dey et al., 2012; Saini et al., 2012). Fluoride contaminated plants showed common morphological symptoms like chlorosis; tip and marginal necrosis are known (Dey et al., 2012; Fornasiero, 2003). Apart from morphological symptoms, F interferes with phosphorylation of phosphoproteins in cellular membranes (Suwalsky et al., 2004), enzyme activities (Fornasiero, 2003), photosynthetic pigments structures and other metabolic processes (Kamaluddin and Zwiazek, 2003). The migration of F from soil to plants is influenced by many factors including soil pH, humus content etc. It is also reported (Stevens et al., 1998a, 1998b) that the ionic F can be easily taken up by the plants roots. Fluoride is usually readily bound to soil surfaces at neutral pH and is not available to plants (Jha et al.,

E-mail address: nkmenvbu@gmail.com.

http://dx.doi.org/10.1016/j.ecoenv.2017.06.009
Received 17 January 2017; Received in revised form 26 May 2017; Accepted 2 June 2017
Available online 07 June 2017

2009). However, plants can accumulate fluoride from contaminated sites through root system (Gao et al., 2012). The F thus absorbed is translocated to the shoots, causing physiological, biochemical and structural damage and even cell death (Dey et al., 2012; Jha et al., 2009). Some plants even accumulate F at higher concentrations (up to 4000 μg Fg^{-1}) without exhibiting any sign of toxicity (Jha et al., 2009). It is well documented that plants can develop, on exposure to excess F concentration either in soil or in solution culture such as germination, root and shoot growth, chlorosis, leaf tip burn, leaf necrosis and reduction of grain yield (Datta et al., 2012; Dey et al., 2012; Maitra et al., 2016). Most other plants, however, show signs of toxicity at much lower concentration while some species are extremely sensitive to concentration < 20 μg g^{-1} (Jha et al., 2009). Chakrabarti et al. (2013) and Gupta et al. (2009) have reported significant accumulation of F in paddy crops irrigated within F contaminated water in a village in West Bengal, India. In the dry season, groundwater is the only source of irrigation. Moreover, rice cultivation mainly depends on underground water which is fluoride contaminated in major rice cultivated areas of West Bengal. This particular aspect explains the importance of F issue in rice (*Oryza sativa* L.). Rice is an important human food crop worldwide and is considered to be the model of monocot species for molecular biology research. All activities related to rice cultivation such as germination, raising of seedling, transplanting etc. in the main field are mostly done with groundwater irrigation.

A number of studies have been reported on F uptake and its effects on different plant species. However, there is not much information available on the effects of F on the early growth and development of plants that are commonly used by farmers in this region of India. Hence, the objective of this study was to understand and determine the phytotoxic effects of F on the early growth phase of seedlings of four varieties of paddy, an important agricultural crop. The findings from this study prove to be important and useful for farmers, agricultural experts and researchers.

2. Materials and methods

2.1. Design of the experiment

Seeds of *Oryza sativa* were procured from Burdwan University seed multiplication farm, Burdwan, West Bengal. The entire laboratory experiment was conducted in the Department of Environmental Science, The University of Burdwan, Burdwan, West Bengal. Three different concentrations of fluoride viz., 0(control), 5, 10 and 20 mg/L F were evaluated on seed germination and seedling growth of four rice varieties viz., MTU-1010, IET-4786, IET-4094 and GB-1. Fluoride was applied as a solution of NaF. The higher concentration (20 mg/L) of fluoride was considered as per the report published in the previous literature. All the experimental rice seeds of the respective varieties were surface sterilized by treating with 1% sodium hypochloride (NaOCl) solution for one minute followed by thorough washing with double distilled water. The seeds were germinated on wet filter paper (Whatman no. 1) in petri plates (dia 90 mm). Each perti dish was covered with a lid and incubated at room temperature (30 °C). Stock solution was prepared by dissolving required amount of sodium fluoride (NaF) in double distilled water to get 100 mg/L fluoride solution. Appropriate dilutions were made to prepare 5, 10 and 20 mg/L NaF concentrations. Control experiments were also set up by using only double distilled water. Each treatment was replicated thrice following complete randomized design (CRD). The seeds were allowed to germinate and grow for 15 days. During the entire experimental period, Petri dishes were periodically moistened with the respective fluoride solution. All the growth parameters were recorded and biochemical tests were performed with 15 day-old seedlings.

2.2. Electrolyte leakage

The membrane damage of plant parts was measured by following the method of Valentoric et al. (2006). The plant samples (root, stem, and leaf) were cut into 1-cm pieces weighing about 1 g each and subsequently dipped into 15 ml deionized water. The tubes were then tightly capped and shaken for 24 h at 25–30 °C. The electrical conductivity (EC1) of the solution was measured with an electrical conductivity meter (HI 8733, Hanna Instruments Inc., Woonsocket, USA). Afterwards, the samples were autoclaved at 120 °C for 20 min and allowed to cool (25 °C) to record the electrical conductivity (EC2) of the solution. The electrolyte leakage is expressed as:

$$EL(\%) = \frac{EC1}{EC2} x100 \quad (1)$$

2.3. Determination of chlorophylls and carotenoids

The chlorophyll and carotenoid level were estimated by following the standard methods (Arnon, 1949; Ikan, 1969).

2.4. Determination of catalase activity

The catalase activity was measured by following the method of Sinha (1972). The activity of catalase was expressed as μM H_2O_2/g protein/min (Tanase et al., 2013).

2.5. Estimation of Moisture content and dry biomass

Moisture content (%) of the plant materials under study was determined according to the method suggested by Kaya (1998). The % moisture was calculated based on the following formula:

$$\%\ of\ moisture = [\frac{(W_0 - W_1)}{W_0}]x100 \quad (2)$$

$Drybiomass = W_0 - W_1$ where, W_0 = Initial Weight W_1 = Final Weight

2.6. Quantification of protein

The protein content in the plant material was determined by the method of Lowry et al. (1951). The concentration of protein was calculated using standard value from a standard curve.

2.7. Estimation of amino acids, carbohydrates and phenols

Spectroscopic method (Moore and William, 1954) was used for the quantification of amino acids. The total carbohydrate was estimated by Anthrone method (McCready et al., 1950). Total phenolics were determined by colorimetric method described by Swain and Hillis (1959) using Folin-Ciocalteu reagent.

2.8. Determination of growth parameters

Ten fully grown almost uniform size seedlings were collected randomly from each treatment and washed gently in distilled water. Tissue paper was used to soak the adhering water. Then the ten plants for each treatment were used for determination of fresh and dry weights. Dry weight was taken after drying the samples for 72 h at 70 °C in an oven.

2.9. Determination of MDA content

leaf tissue (0.1 mg) was homogenized by adding 10 ml 0.1% (w/v) TCA. The homogenate was centrifuged for 10 min (15,000 × *g*, 4 °C).