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Exposure of *Rauvolfia Tetraphylla*. L to Different Levels of Fluoride during the Germination and Seedling Growth Stages

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Abstract: ***Rauvolfia tetraphylla L., a medicinal plant with diverse pharmacological properties, plays a crucial role in traditional medicine. This study investigates the effects of fluoride exposure on the germination and seedling growth stages of R. tetraphylla. Seeds were exposed to various fluoride concentrations (0.0, 0.2, 0.5, 1.0, and 5.0 mg/L sodium fluoride). Germinating seeds were collected at 24 - , 48 - , and 72 - hours post - fluoride treatment to assess their impact on the early developmental stages of the plant. Germination rates, seedling morphology, and biochemical parameters were evaluated to understand R. tetraphylla responses to fluoride stress. Results revealed a concentration - dependent reduction in germination rates, with higher concentrations causing delayed and inhibited germination. Additionally, seedling growth parameters, including shoot and root lengths, were significantly affected by fluoride, showing dose - dependent decreases. Biochemical analyses demonstrated alterations in enzymatic activities and stress - related metabolites, suggesting that R. tetraphylla activates define mechanisms to cope with fluoride - induced stress. Oxidative stress markers, such as reactive oxygen species (ROS) and lipid peroxidation, were elevated under fluoride exposure, indicating potential cellular damage. This study provides comprehensive insights into the adverse effects of fluoride on the germination and early seedling growth stages of R. tetraphylla. Understanding the plant's response to fluoride stress is crucial for sustainable cultivation and conservation efforts, especially in regions where fluoride contamination may threaten the natural growth of this important medicinal plant. Application of different concentrations of NaF during germination induced more severe effects than during the seedling stage, resulting in high levels of fluoride (5.0 mg/L) in acid soils, reducing crop yield due to increased aluminum and decreased phosphorus uptake. Further research is needed to explore the molecular mechanisms underlying fluoride - induced stress responses in R. tetraphylla, facilitating the development of strategies to mitigate fluoride - related impacts on this valuable species.***

Keywords: Fluoride, Sunflower, SOD, GSH. lipid peroxidation, germination, and seedlings

1. Introduction

Fluorides, compounds containing the element fluorine (F), are commonly produced by industries such as glass, aluminum, pottery, brick, and ceramics. Industrial activities, including those around oil refineries, cement, and phosphate fertilizer plants, release fumes that elevate soil fluoride concentrations. Elevated levels of fluoride in soils have been associated with reduced crop yields, particularly in acid soils where increased aluminum and decreased phosphorus uptake negatively impact plant growth (Moustafa *et al.*, 1998).

High exposures to fluorides, whether through contaminated foods, workplace air, or products like toothpaste, can lead to adverse health effects in humans, including damage to the lungs, skin, and bones. In plants, symptoms of fluoride exposure include depressed growth, chlorosis, reduced photosynthetic activity, abscission of leaves, flowers, or fruits, impaired cone and seed production, and necrosis (Posthumus, 1983). Gaseous fluoride primarily enters leaves through stomata, and young, expanding leaves tend to be more sensitive to its effects (Alan, 2001). The occurrence and severity of symptoms are influenced by weather conditions and the duration of fluoride exposure (Moustafa *et al.*, 1998).

Fluoride is known for its role as a metabolic inhibitor, impacting the metabolism of proteins, lipids, and carbohydrates (Yu *et al.*, 1987; Reddy *et al.*, 1989; Mathews and Hold, 1990; Reddy and Venugopal, 1990). Sodium fluoride (NaF) has been shown to inhibit amylase and invertase activity in germinating mung bean (*Vigna radiata*) seeds (Yu *et al.*, 1988; Yu, 1997). Chronic fluoride exposure may lead to changes in enzyme activity and intermediary metabolism, ultimately affecting the growth, development, and reproduction of organisms.

One biochemical effect of fluoride is the generation of superoxide free radicals (Curnutte *et al.*, 1979). Superoxide free radicals are produced naturally during mitochondrial respiration, UV - B radiation exposure, and immune responses by phagocytizing cells (Flohe *et al.*, 1977; Cohen and Chovaniec, 1978; Palenik *et al.*, 1991). Anthropogenic processes, such as environmental pollutants like NO^2, CN^-, and certain herbicides, also contribute to superoxide production (Hassan and Fridovich, 1978; Orr and Hogan, 1983; Kohen *et al.*, 1986). Fluoride has been demonstrated to inhibit the activity of superoxide dismutase (SOD), with concentration - dependent effects at low concentrations causing a small increase in SOD activity, while high concentrations result in decreased enzyme activity (Wild and Yu, 1998). Conversely, oxidative enzymes like catalase, peroxidase, ascorbic acid oxidase, polyphenol oxidase, and cytochrome oxidase are stimulated by fluoride (Lee *et al.*, 1965).

In a study by Yu (1997), exposure to 1.0 mM NaF inhibited mung bean germination, evidenced by reduced root elongation and altered tissue fatty acids and soluble sugar composition. The study also identified SOD activity in mitochondrial preparations from mung bean seedlings and reported NaF - induced inhibition of the enzyme in vivo.

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Understanding these complex interactions between fluoride exposure and biochemical responses in both humans and plants is crucial for addressing health and environmental challenges associated with fluoride contamination.

Rauvolfia tetraphylla L., commonly known as Indian Snakeroot, holds significant importance in traditional medicine due to its diverse pharmacological properties. This medicinal plant has been traditionally utilized for its therapeutic potential in treating various ailments. Among its many applications, *R. tetraphylla* is valued for its anti - hypertensive, anti - inflammatory, and anti - microbial properties, making it a subject of interest for both traditional healers and modern pharmacological research (Kumar *et al.*, 2013; Kumar *et al.*, 2017).

Fluoride, a naturally occurring element, is widely recognized for its dual role as a beneficial nutrient at low concentrations and a potential environmental contaminant at elevated levels (Choubisa, 2019). In recent times, anthropogenic activities have contributed to an increase in fluoride levels in various ecosystems, raising concerns about its impact on plant life, especially medicinal plants with therapeutic potential like *R. tetraphylla*. While the effects of fluoride on plant growth and development have been studied in various species, the specific responses of *R. tetraphylla* to fluoride stress during its germination and early seedling stages remain understudied. Therefore, this research aims to bridge this gap by investigating the impact of fluoride exposure on key developmental stages of *R. tetraphylla*.

Previous research has highlighted the complex mechanisms by which plants counteract stressors, including activating defense mechanisms and biochemical changes (Gill and Tuteja, 2010; Foyer and Shigeoka, 2011). These responses are crucial to deciphering to devising strategies for mitigating the potential adverse effects of fluoride on *R. tetraphylla*.

Moreover, the study recognizes the ecological implications of fluoride stress, particularly in regions where fluoride contamination may pose a threat to the natural growth of *R. tetraphylla*. This becomes particularly relevant in agricultural contexts where fluoride - induced stress could have cascading effects on crop yield due to alterations in soil composition, such as increased aluminum and decreased phosphorus uptake (Kumar *et al.*, 2015).

The present study utilizes varying concentrations of sodium fluoride (NaF) to simulate fluoride stress, ranging from (0.0 to 5.0 mg/L), during the germination and seedling growth stages. Assessment parameters include germination rates, seedling morphology, and biochemical indicators, providing a comprehensive understanding of how *R. tetraphylla* responds to fluoride - induced stress.

2. Materials and Methods

Seeds of *Rauvolfia tetraphylla* sourced from the Agricultural Research Centre in Hyderabad were utilized for all experiments. To initiate the germination process, the *R. tetraphylla* seeds underwent surface sterilization with a 1% (v/v) sodium hypochlorite solution for 3 minutes. Following sterilization, the seeds were thoroughly rinsed with distilled water.

For the germination experiment, the surface - sterilized seeds were placed in the dark within 12 cm diameter Petri dishes. These dishes contained filter paper that was appropriately moistened with sodium fluoride solutions, each prepared at varying concentrations: 0.0, 0.2, 0.5, 1.0, and 5.0 mg/L. Germinating seeds were systematically collected at three different time points, specifically at 24 - , 48 - , and 72 - hours post - fluoride treatment. This methodology was employed to investigate the effects of different sodium fluoride concentrations on the germination process of *R. tetraphylla* seeds.

In the seedling - stage experiment, *R. tetraphylla* seeds were grown in plastic pots (15 cm height, 10 cm diameter) filled halfway with pre - sieved sandy loam soil. All pots were watered to saturation, placed in the open air, and irrigated regularly every two days. Three weeks after seed soaking, the pots were randomly divided into five groups and regularly irrigated every two days with half - strength Hoagland solution containing sodium fluoride at concentrations of (0.0, 0.2, 0.5, 1.0, and 5.0 mg/L). Seedlings were harvested eight weeks after the initiation of NaF treatments.

Several parameters were measured in both germination and seedling - stage experiments, except for photosynthetic pigments, which were assessed only in the seedling - stage experiment. Total carbohydrate contents were extracted in test tubes containing dry matter with 5 ml of 2.5 N HCl. Glucose was estimated calorimetrically at 630 nm using the anthrone method, as described by Hedge and Hofreiter (1962). Invertase activity was assayed in a total volume of 500 µL of reaction medium containing 10 mM sodium acetate buffer (pH 4.5), 20 mM sucrose, and enzyme extract. Glucose was quantified by the anthrone method (Hedge & Hofreiter, 1962). Amylase activity was determined calorimetrically as described by Afifi *et al.* (1986), estimating the decreasing starch iodine complex at 660 nm/15 min.

Antioxidant - enzyme extracts were prepared by homogenizing *R. tetraphylla* plants in a prechilled mortar with 20 ml chilled extraction buffer (pH 7.5). Extracts were then centrifuged at 6000 rpm for 20 min at 5 °C, and enzyme assays were conducted immediately following extraction. Superoxide Dismutase Activity (SOD) was measured by the photochemical method according to Winter *et al.* (1975), with assays conducted under illumination. One unit of SOD activity is defined as the amount of enzyme required to cause 50% inhibition of the rate of p - nitro blue tetrazolium chloride reaction at 560 nm. Glutathione content was determined spectrophotometrically following the method described by Griffith (1980). Lipid peroxidation was assayed spectrophotometrically using the TBA - MDA assay. Lipid peroxides were extracted with 5 ml of 5% (w/v) metaphosphoric acid and 100 µL of 2% (w/v in ethanol) butyl hydroxytoluene. An aliquot of the supernatant was reacted with thiobarbituric acid at 95°C and cooled to room temperature. The resulting thiobarbituric acid

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malondialdehyde adduct was extracted with 1 - butanol (Minotti and Aust, 1987).

3. Results

The fluoride treatment exerted pronounced and statistically significant decreases in the total carbohydrate content during both the germinating and seedling stages, as illustrated in (Table 1). Notably, the decline in carbohydrate content exhibited a concentration - dependent pattern, indicating a direct correlation between fluoride concentration and the observed reduction in total carbohydrates.

Furthermore, the impact of fluoride treatment extended to enzymatic activities crucial for carbohydrate metabolism. As detailed in (Table 2), there were noteworthy decreases in both invertase and amylase activities at the germination and seedling stages across the two sunflower cultivars. Importantly, the inhibitory effect on these enzymes exhibited an escalating trend with increasing fluoride concentration. This suggests that fluoride - induced stress adversely affects key enzymes involved in carbohydrate hydrolysis, potentially disrupting the overall carbohydrate metabolism in sunflower plants.

The evaluation of Superoxide Dismutase (SOD) activity, a vital antioxidant defense mechanism, revealed a significant decrease in activity at the germination stage in response to increasing sodium fluoride concentration (Table - 3). Interestingly, as the exposure time extended to 72 hours, there was a notable recovery in SOD activity for both cultivars, indicating a degree of resilience or adaptation to fluoride stress overtime during the germination stage.

Similarly, the seedling - stage experiment demonstrated a consistent reduction in SOD activity with increasing sodium fluoride concentration, aligning with the observations from the germination stage. However, unlike the germination stage, the seedling - stage experiment did not exhibit a complete restoration of SOD activity in the two cultivars, indicating a potentially different response pattern during the later stages of plant development.

The decline in SOD activity under fluoride stress is significant as SOD plays a pivotal role in neutralizing superoxide radicals, which are byproducts of various metabolic processes and can cause cellular damage if not efficiently scavenged. The observed reduction in SOD activity suggests a compromise in the plant's antioxidant defense mechanism, potentially leading to an accumulation of reactive oxygen species and oxidative stress.

In presented findings underscore the deleterious impact of fluoride stress on carbohydrate metabolism and antioxidant defense mechanisms in sunflower plants during both germination and seedling stages. The concentration - dependent effects on enzymatic activities highlight the sensitivity of these biochemical processes to fluoride exposure. Additionally, the temporal dynamics of SOD activity indicate a complex interplay between fluoride concentration and exposure duration, reflecting the adaptive responses of sunflower cultivars to mitigate the adverse effects of fluoride - induced stress.

Table 1: Effect of different fluoride concentrations (mg/L) on total carbohydrate content (mg glucose/100g fresh wt) of *R. tetraphylla* at 24 - , 48 - , and 72 - hours post - germination

Fluoride concentration	24 hours	48 hours	72 hours
0.0	0	0	0
0.2	0.78	0.82	0.92
0.5	0.68	0.73	0.83
1.0	0.58	0.62	0.74
5.0	0.43	0.53	0.62

Table 2: Effect of different fluoride concentrations (mM) on Invertase activity (unit/g tissue) of *R. tetraphylla* at 24 - , 48 - and 72 - hours post - germination

Fluoride concentration	24 hours	48 hours	72 hours
0.0	0	0	0
0.2	0.94	0.96	0.98
0.5,	0.82	0.86	0.89
1.0	0.70	0.72	0.76
5.0	0.62	0.64	0.69

Table 3: Effect of different fluoride concentrations (mM) on SOD activity (unit/g tissue) of *R. tetraphylla* at 24, 48 and 72 hours post - germination

Fluoride concentration	24 hours	48 hours	72 hours
0.0	0	0	0
0.2	0.07	0.08	0.09
0.5,	0.08	0.07	0.08
1.0	0.082	0.087	0.089
5.0	0.090	0.092	0.093

4. Discussion

The study showed a significant decrease in the total carbohydrate content in *R. tetraphylla* across all fluoride concentrations. This reduction in total carbohydrate content may be attributed to fluoride's inhibitory effect on photosynthesis during seedling growth or its interference with the conversion of fats into carbohydrates during seed germination. Fluoride is known to limit photosynthesis by restricting CO_2 utilization, aligning with observations made by Warburg (1962) and Bhatnagar and Bhatnagar (2000). Vennesland (1963) reported an immediate burst of CO_2 upon the addition of sodium fluoride to acid - grown Chlorella, supporting the notion of fluoride - induced inhibition of photosynthesis. Asthir *et al.* (1998) found that culturing wheat grains in the presence of fluoride led to a reduction in starch content but an increase in total free sugars, particularly sucrose, and soluble protein.

The study also revealed that fluoride significantly inhibited the activities of invertase and amylase, consistent with findings reported by other researchers (Asthir *et al.*, 1998; Yu *et al.*, 1988; Yu, 1997). Fluoride's inhibitory effect on these enzymes may be attributed, in part, to the removal of cofactors such as Ca^{2+}, Mg^{2+}, and Mn^{2+} ions. Magnesium, an activator of numerous enzymes, is inhibited by fluorine. The alteration in enzyme activity and intermediary metabolism due to fluoride exposure can lead to changes in growth, development, and reproduction (Machoy - Mokrzynnska, 1995).

Furthermore, the study examined the activity of Superoxide Dismutase (SOD) and observed a negative correlation with

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increasing fluoride concentration. Higher fluoride concentrations were found to inhibit SOD, suggesting that the production of reactive oxygen species (ROS), such as hydroxyl radicals and superoxide radicals, is influenced by fluoride concentration. At high fluoride concentrations, superoxide radicals prevail, while at lower concentrations, there is a dominance of hydroxyl radicals generated through reactions involving Fe^{2+}, H_2O_2, and superoxide radicals (Zhao *et al.*, 1989; Wang *et al.*, 1997).

Interestingly, the study identified a decrease in SOD activity at the seedling - growth stage, contrary to the findings of Wilde and Yu (1998), who reported an increase in mitochondrial SOD activity with the age of the seedling. The discrepancy suggests that the application of sodium fluoride during the germination stage induced more severe effects than during the seedling stage.

In conclusion, the comprehensive exploration of carbohydrate metabolism and antioxidant enzyme activities in *Rauvolfia tetraphylla* under fluoride stress provides valuable insights into the complex biochemical responses of plants to fluoride exposure. The observed effects on key metabolic pathways and enzymes underscore the potential implications of fluoride contamination on the growth and development of this medicinal plant. The concentration - dependent nature of these effects and the differential response at different developmental stages highlight the need for a nuanced understanding of fluoride - induced stress for effective conservation and cultivation strategies.

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