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Mutagenic Impact of Sodium Azide on Growth and Development in Spinach (Spinacia oleracea L.)

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Abstract: *Spinacia oleracea L. (spinach) is a nutritionally rich leafy vegetable with significant medicinal and agricultural value. This study examines the mutagenic effects of sodium azide (NaN□) on spinach growth and development by assessing seed germination rates, shoot and root growth. Seeds were treated with varying concentrations of NaN□ (0.001%–0.005%) and observed over 8, 16, and 24 hours. Results revealed a dose-dependent decline in germination, decreasing from 94% (control) to 9% at 0.005% NaN□ after 24 hours. Similarly, shoot and root lengths were significantly reduced, with shoot length declining from 7.2 cm (control) to 1.4 cm, and root length from 1.5 cm to 0.4 cm at the highest concentration. While some phytochemical variations were observed, NaN□ primarily exhibited inhibitory effects on growth. These findings suggest that while controlled doses of NaN□ may induce genetic variability beneficial for crop improvement, careful application is necessary to mitigate its adverse effect.*

Keywords: Spinacia oleracea L., sodium azide (NaN□), mutagenic effects, seed germination

I. INTRODUCTION

Spinach (*Spinacia oleracea* L.) is an annual, leafy vegetable belonging to the family Chenopodiaceae for its high nutritional value, rich in vitamins, minerals, and antioxidants. It plays a crucial role in human health and is a staple in many diets worldwide, spinach holds significant medicinal value in various traditional systems, including Ayurveda, Unani, and Siddha. It has been utilized for its cooling, laxative, and anti-inflammatory properties, as well as its role in treating ailments such as urinary disorders, respiratory issues, and skin conditions. (Khare, 2007). However, environmental factors, genetic modifications, and exposure to mutagens can significantly influence its growth and development.

Mutagens are physical or chemical agents that cause genetic mutations, leading to alterations in plant growth, morphology, and overall productivity. Understanding the impact of mutagens on spinach is essential, as it helps in evaluating genetic stability, potential improvements in crop yield, and resistance to environmental stressors. Among chemical mutagens, sodium azide (NaN_3) has gained importance due to its ability to induce point mutations, potentially leading to improved agronomic traits. Sodium azide interacts with plant DNA, causing A-T to G-C transitions, which can result in modified protein functions, leading to increased stress tolerance, enhanced growth, and improved yield.

Spinach (*Spinacia oleracea*) is a highly nutritious leafy vegetable with significant medicinal and agricultural value. Research has highlighted its antimicrobial peptides (Segura et al., 1998), flavonoid composition and storage stability (Sara et al., 2005), and the development of low-oxalate mutants (Murakami et al., 2009). Its pharmacological properties include antioxidant, anti-inflammatory, and hepatoprotective effects (Otari et al., 2010; KO et al., 2014). Studies on bacterial colonization of spinach have provided insights into its microbiome (Gabriela et al., 2013).

Phytochemical investigations have confirmed the presence of flavonoids, phytosterols, and other bioactive compounds contributing to its therapeutic potential (Chaudhari et al., 2015; Gutierrez et al., 2019). Genetic and breeding studies have traced its ancestry to *S. turkestanica* and explored its resistance to diseases such as downy mildew (Ribera et al., 2021; Gehendra&Ainong, 2021). Spinach extracts have shown potential in stress adaptation and dietary supplementation (DO et al., 2020). Agronomic studies reveal that organic manure improves spinach growth and yield (Parwada et al., 2020), and its use as green manure enhances soil fertility and crop productivity (Kim et al., 2023).

Additionally, species authentication techniques using HPTLC and HPLC-PDA methods have differentiated *S. oleracea* from other spinach varieties (Kureshi et al., 2023).

Overall, spinach is a highly valuable crop with applications in nutrition, medicine, and sustainable agriculture. This study aims to evaluate the mutagenic effects of sodium azide on the growth and development of *Spinacia oleracea* L. By analysing parameters such as seed germination rate, root and shoot length, and phytochemical content, this research seeks to determine how mutagenic exposure influences *spinach* growth. The findings could have significant implications for plant breeding, agricultural productivity, and food security.

Photo plate 1: Plant of *Spinacea oleracea*

II. MATERIAL AND METHODS

The current study is designed for the mutagenic effects on seeds of selected plant species (*Spinacia oleracea* L.) which will be as per the following steps and methods. Chemical mutagenic methods were preferred with Sodium azide (NaN3) for current research work in combination of various modified treatments methods given by Gabriela et al. (2013), Chaudhari et al (2015), Raughani and Miri (2019), Ribera *et al* (2021), Kim et al (2023).Different concentration of mutagen aqueous to mutagenic can be used in effect are as follows.Control, 0.001%, 0.002%, 0.003%, 0.004% and 0.005%**.** After treatment of specific mutagen seeds were grown in garden soil for further observational parameters.

III. RESULT AND DISCUSSION

Seed germination:

Based on programmed methodology result will be recorded in graphical representation for mutagenic effect on seed treatment of *Spinacia oleracea L.*

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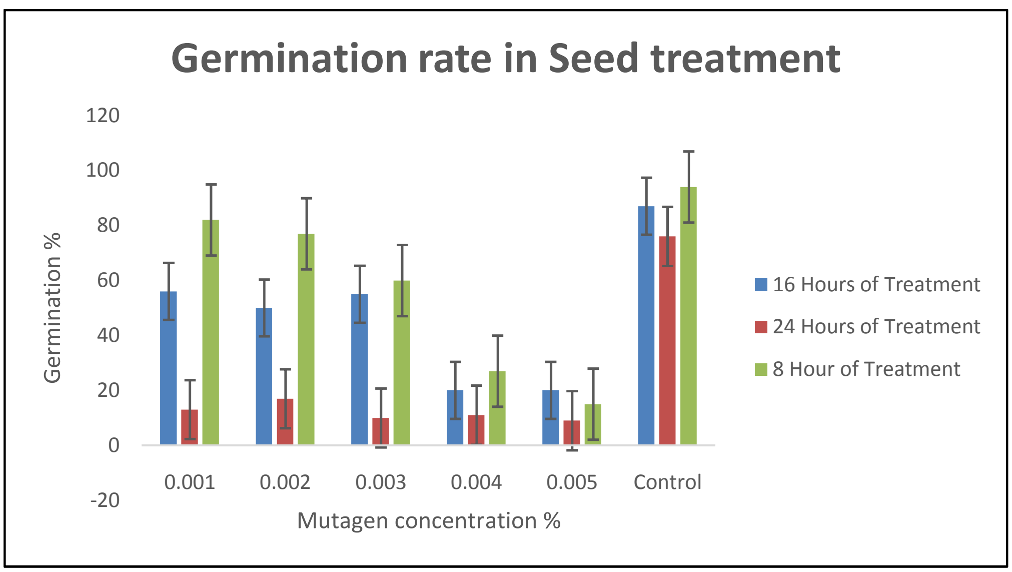
Photo plate 2: Seed germination of *S. oleracea*



Graph 1: Germination rate of seed of *Spinacia oleracea* treated with mutagen

Observations on the seed treatment of *Spinacia oleracea* L. with varying mutagenic concentrations reveal a clear trend of decreasing germination rates as the concentration increases. A control group, maintained under standard conditions without any mutagen treatment, served as a baseline for comparison. At 8 hours of treatment, the control group exhibited a germination rate of 94%. However, increasing the mutagenic concentration resulted in a progressive decline

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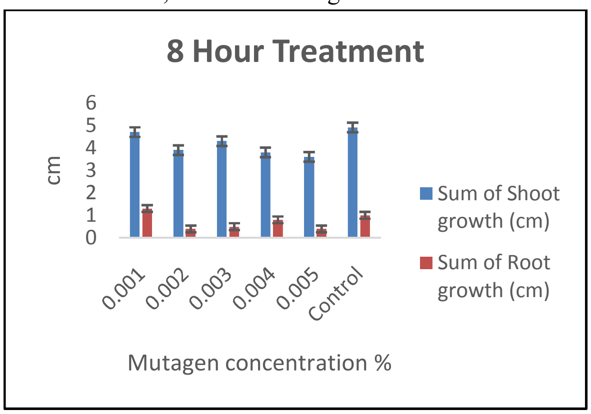
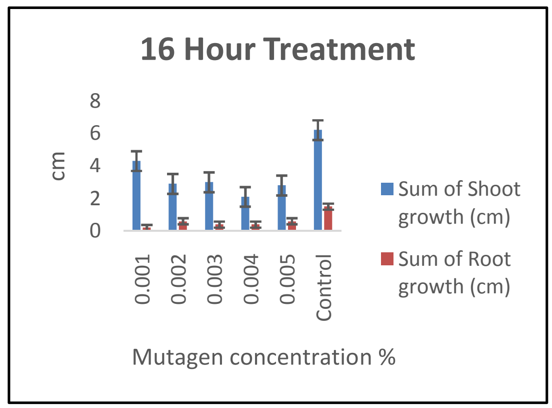
in germination rates, with 0.001% concentration reducing it to 82%, 0.002% to 77%, 0.003% to 60%, 0.004% to 27%, and 0.005% to just 15%.

At 16 hours of treatment, the control group's germination rate dropped slightly to 87%, while the mutagen-treated seeds showed a significant decline. At 0.001% concentration, the germination rate was 56%, decreasing further to 50% at 0.002%, 55% at 0.003%, and 20% at both 0.004% and 0.005% concentrations. By 24 hours of treatment, the control group showed a germination rate of 76%, but increasing mutagenic concentrations further inhibited germination. The rates were recorded as 13% at 0.001%, 17% at 0.002%, 10% at 0.003%, 11% at 0.004%, and the lowest, 9%, at 0.005%. These results indicate that higher concentrations of mutagen have a detrimental effect on seed germination, with prolonged exposure further exacerbating the decline in germination rates.

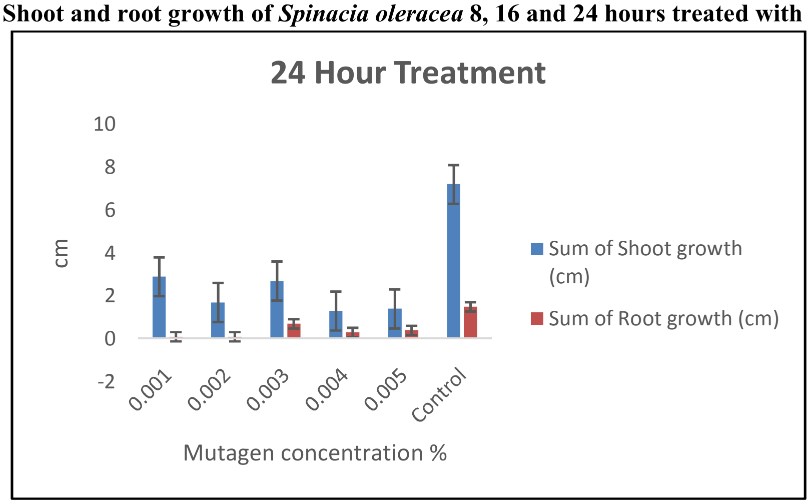
Mutagen effect on growth of *Spinacia oleracea L.*

In the seed treatment experiment with *Spinacia oleracea* L. and different mutagenic concentrations, the following observations were made:





Graph: Shoot and root growth of *Spinacia oleracea* 8, 16 and 24 hours treated with mutagen



Graph 4: Shoot and root growth of *Spinacia oleracea* 24 hour treated with mutagen

Observations on the effects of mutagenic concentrations on the shoot and root growth of *Spinacia oleracea* L. can be summarized as follows: In the 8-hour treatment, the control group exhibited shoot growth of 4.9 cm and root growth of 1 cm. As the mutagenic concentration increased, both shoot and root growth showed fluctuations. At 0.001%, shoot growth slightly decreased to 4.7 cm, while root growth increased to 1.3 cm. With 0.002%, shoot growth dropped to 3.9 cm, and root growth declined to 0.4 cm. At 0.003%, shoot growth increased to 4.3 cm, while root growth remained low

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at 0.5 cm. Higher concentrations (0.004% and 0.005%) resulted in reduced shoot growth (3.8 cm and 3.6 cm, respectively) and varying root growth (0.8 cm and 0.4 cm).

After 16 hours of treatment, the control group showed shoot growth of 6.2 cm and root growth of 1.5 cm. As the mutagenic concentration increased, both shoot and root growth declined. At 0.001%, shoot growth dropped to 4.3 cm, and root growth reduced significantly to 0.2 cm. At 0.002%, shoot growth declined further to 2.9 cm, while root growth increased to 0.6 cm. At 0.003%, shoot growth slightly increased to 3.0 cm, but root growth remained at 0.4 cm. At higher concentrations (0.004% and 0.005%), shoot growth was further reduced (2.1 cm and 2.8 cm), with root growth fluctuating between 0.4 cm and 0.6 cm.

In the 24-hour treatment, the control group had the highest shoot (7.2 cm) and root (1.5 cm) growth. Increasing concentrations of mutagen had a significant inhibitory effect. At 0.001%, shoot growth dropped to 2.9 cm, with root growth at 0.1 cm. At 0.002%, shoot growth declined to 1.7 cm, with root growth remaining at 0.1 cm. At 0.003%, shoot growth slightly improved to 2.7 cm, while root growth increased to 0.7 cm. However, at 0.004% and 0.005%, shoot growth fell to 1.3 cm and 1.4 cm, respectively, with root growth at 0.3 cm and 0.4 cm.

Overall, the results indicate that increasing mutagenic concentrations had a generally detrimental effect on the growth of *Spinacia oleracea* L., with both shoot and root growth showing a decreasing trend as exposure time and concentration increased.

IV. CONCLUSION

The study demonstrated that increasing mutagenic concentrations of NaN_3 negatively affected the germination, shoot, and root growth of *Spinacia oleracea* L. Higher concentrations led to a significant decline in seed germination and seedling development, with the highest concentration (0.005%) reducing germination to 9% and shoot growth to 1.4 cm after 24 hours. While some phytochemical variations were observed, the overall impact was detrimental. These findings suggest that controlled doses of NaN_3 may induce genetic variability in spinach, potentially aiding in quality improvement while requiring careful application to minimize adverse effects.

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