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Determining the Effect of Chemical Mutagens on Vegetative Growth of *Calendula officinalis* L. (cv. Calypso Orange)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The present investigation was carried out at the laboratory of floriculture and experimental farm of Mata Gujri college, Fatehgarh Sahib, Punjab. The experiment was laid out in randomized block design (RBD) with nine treatments and three replications. The treatments were T_1 i.e., Sodium azide @1500ppm, T_2 i.e., Sodium azide @2500ppm, T_3 i.e., Sodium azide @3500ppm, T_4 i.e., Sodium azide @4500ppm, T_5 i.e., Diethyl sulphate @1500ppm, T_6 i.e., Diethyl sulphate @2500ppm, T_7 i.e., Diethyl sulphate @3500ppm, T_8 i.e., Diethyl sulphate @4500ppm and T_9 i.e., control. Observations were recorded for vegetative among all treatments maximum plant height (24.84cm), number of leaves per stem (8.95), number of stems per plant (7.48), stem length (22.85cm), leaf length (9.48cm), plant spread (24.89cm^2) and diameter of main stem (9.39mm) were found

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maximum in T_1 i.e., Sodium azide @1500ppm. Best vegetative parameters were recorded in Sodium azide @1500ppm Therefore, Sodium azide @1500ppm is best dosage for *Calendula officinalis* cv. Calypso orange. From the present study it can be concluded that the lowest concentration of Sodium Azide (1500ppm) reported the best performance of vegetative parameters such as plant height, number of leaves per stem, number of stems per plant, stem length, leaf length, plant spread, and diameter of main stem. From the results, it can be concluded that Sodium Azide @1500ppm is best dosage for *Calendula officinalis* cv. Calypso orange.

Keywords: Calendula; chemical mutagens; calypso orange; growth parameters.

1. INTRODUCTION

In recent decades, the floriculture business has grown significantly. A breeder goals are always to generate new and unusual cultivars of ornamentals, and this process is never-ending (Datta, 2009). Pot marigold, or *Calendula officinalis* L., belongs to the Asteraceae family and has a chromosomal number of 2n= 32. The plant's vegetative sections are green, and the stems have small hairs covering them. According to Filipovic et al., (2016), the flower's colour varies from yellow to orange. The flower contains disc and ray florets, which may open up in the spring [1].

The herb *Calendula officinalis* is native to southern Europe and the Eastern Mediterranean region. It can be an annual or short-lived perennial. It is a popular garden escape in cold, temperate areas and has been cultivated for a very long time. It has been utilized for a variety of gastronomic and therapeutic uses for many ages. Common names for this species include pot marigold, English marigold, and Scotch marigold [2-4]. It is important to distinguish this species from marigolds, which are a group of numerous 'Tagetes' species. Field marigold is a branching annual plant native to southern Europe. Its daisies are one inch in size and are either yellow or orange in colour [5,6].

The herbaceous plant *Calendula officinalis* reaches a height of 30 to 60 cm. The plant is heterophyllous in a single individual, with lanceolate upper and lower leaves, and an inflorescence resembling a basket with two distinct types of flowers. Its distinctive features include ligular blooms on the outside and tubular flowers on the inside [7-9].

Using chemical mutagens like Sodium azide and Diethyl sulphate for calendula flowers have specific benefits and advantages in the process of inducing mutations for breeding purposes such as increased genetic variation, efficiency, targeted mutagenesis and compatibility with breeding programs. The objective of this study was to study the effect of chemical mutagens on vegetative growth of *Calendula officinalis* (Calypso orange).

2. MATERIALS AND METHODS

The present investigation was conducted at Research Farm, Mata Gujri College, Fatehgarh Sahib, Punjab during 2023-2024. Field of experimental site lies at 30.6435° North latitude and 76.3970° East longitudes. The altitude of the location is 246 meter above the mean sea level. The climate that prevails in Fatehgarh Sahib is sub-tropical. The area involves maximum temperature between 10.2°C and 35.60°C in the summer and lowest temperature between 4.50°C and 20.10°C in the winter. Winter time brought a lot of fog. The average humidity recorded was 89.95%, while the minimum relative humidity was 53.45%. Seeds of the calendula flower cv. Calypso orange was purchased from the Biocarve seeds, Dhablan (Patiala).

2.1 Experimental Design

The experiment was laid out in Randomized Block Design (RBD) with three replications. The seedlings of uniform size (3-5cm) was treated (after 25 days from germination) used for treatment of chemicals on vegetative growth and then planted in the beds with a spacing of 25 cm plant to plant and 30 cm row to row during November 2023.

2.2 Experimental Details

There were 9 treatments of chemicals dosages which were applied to calypso orange cultivar of calendula. The chemicals of Sodium azide and Diethyl Sulphate was purchased from Avra Synthesis private limited, Hyderabad, Telangana, India. The treatments were T_1 i.e., Sodium azide

@1500ppm, T_2 i.e., Sodium azide @2500ppm, T_3 i.e., Sodium azide @3500ppm, T_4 i.e., Sodium azide @4500ppm, T_5 i.e., Diethyl sulphate @1500ppm, T_6 i.e., Diethyl sulphate @2500ppm, T_7 i.e., Diethyl sulphate @3500ppm, T_8 i.e., Diethyl sulphate @4500ppm and T_9 i.e., control.

3. RESULTS AND DISCUSSION

3.1 Plant Height (cm)

The maximum plant height was observed (Table 1) in T_1 i.e., @1500ppm Sodium Azide (24.84cm) which was statistically superior than other treatments. At low concentration of Sodium azide increase in the rate of cell elongation or division as well as auxin activation may be responsible for this mutagen's effect. This finding was similar with Zaka et al*.,* (2004) and Joshi et al. [10]. T_4 i.e., Sodium Azide @ 4500 ppm recorded minimum plant height (21.03cm) of calendula cv. calypso orange which was statistically at par with T_6 (21.86 cm @2500 ppm Diethyl Sulphate) due to the physiological harm brought on by Sodium azide and its hydrolysis products may be the cause of these outcomes at higher concentration of sodium azide. The present study's findings are also in line with observations made *Asparagus densiflorus* by Asrar and El- Nashar [11].

3.2 Number of Leaves Per Stem

There was significant effect on the number of leaves per stem of calendula cv. calypso orange in the Table 1. The T_1 treatment i.e., @1500ppm Sodium Azide (8.95) produced maximum number of leaves per stem on calendula cv. calypso orange which was statistically at par with T_2 i.e., Sodium Azide @2500ppm (8.33), T_3 i.e., Sodium Azide@3500ppm (8.82), T_4 i.e., Sodium Azide @4500ppm (8.86), T_5 i.e., Diethyl Sulphate @1500ppm (8.55), T_6 i.e., Diethyl Sulphate @2500ppm (8.48), T_7 i.e., Diethyl Sulphate @3500ppm (8.87) and T_9 i.e., control (8.38) due to the low concentration of sodium azide the number of leaves per stem was increased due to increase the metabolic activities of plants, and it also enhance nutrient absorption and photosynthesis process. The same results were also reported by Mohamed et al*.,* 2019. Minimum number of leaves per stem was discovered in T_8 i.e., Diethyl Sulphate @ 4500ppm (7.58) which was statistically inferior. The higher concentration of chemical mutagen is toxic for the calendula plant that can cause severe damage to plant cells and disrupt essential biological processes. The same findings were also stated by Gvozdenvoic et al*.* [12] on the sunflower.

3.3 Number of Stems Per Plant

The maximum number of stems per plant was recorded (Table 1) in T_1 i.e., @1500ppm Sodium Azide (7.48) which was statistically at par with T_5 i.e., Diethyl Sulphate @2500pmm (7.12), T_7 i.e., Diethyl Sulphate @ 3500ppm (7.25), and T_9 i.e., control (7.08). These results were observed might be due to the physiological effects of sodium azide and the products of their hydrolysis may be responsible for these results, as well as the reason for the increase in stem count at low concentration of chemical mutagen. The result was closely related with El-Nashar [13]. The minimum number of stems per plant was found in T_8 i.e., Diethyl Sulphate @4500ppm (6.50) which was statistically at par with T_2 (6.88) i.e., Sodium Azide @2500ppm, T_4 (6.72) i.e., Sodium Azide @4500ppm, and T_6 (6.72) i.e., Diethyl Sulphate @2500ppm. Diethyl sulphate is a powerful mutagen at higher concentration it can cause changes in the DNA of plants, disrupt the cell division, hormonal balance with chemical mutagens on plant genetics and development. In connection with this, Sharma et al*.,* 2023 also report the same conclusion.

3.4 Stem Length (cm)

This data shows significant effect of chemical mutagens on the stem length of calendula cv. calypso orange in Table 1. The maximum stem length was observed in T_1 i.e., Sodium Azide @1500 ppm Sodium Azide (22.85 cm) which was statistically at par with T_4 i.e., Sodium Azide @4500ppm (21.85cm) and T_7 i.e., Diethyl Sulphate @3500ppm (21.94cm). These results might be due to effect of the mutagen, resulting in a higher rate of cell elongation or division, could be linked to either auxin or cytokinin at low concentration of chemical mutagen. The same study was reported by Khan et al*.* [14]. The shortest stem length per plant was observed T_8 i.e., Diethyl Sulphate@ 4500ppm (20.01cm). Diethyl sulphate due to the higher concentration of Diethyl sulphate interference with cell elongation pathways and hormonal balance was highlighted in the study as a major factor contributing to the decrease in stem length and this chemical can also disrupt plant respiration by inhibiting cytochrome oxidase, an enzyme crucial for the electron transport chain in mitochondria. As discovered and reported by Gupta et al*.* [15].

Table 1. Effect of chemical mutagens on vegetative growth of *Calendula officinalis* L. (cv. Calypso orange)

Treatments	Plant height(cm)	Number of leaves per stem	Number of stems per plant	Stem length (cm)	Leaf length (cm)	Plant spread (cm^2)	Diameter of main stem (mm)
T_1 Sodium azide@1500ppm	24.84	8.95	7.48	22.85	9.48	24.89	9.39
T_2 Sodium azide@2500ppm	22.23	8.33	6.88	21.38	8.23	21.02	7.52
T_3 Sodium azide@3500ppm	22.85	8.82	7.03	21.61	8.62	22.43	8.35
T_4 Sodium azide@4500ppm	21.03	8.86	6.72	21.85	8.12	21.79	7.97
T_5 Diethyl sulphate@1500ppm	23.48	8.55	7.12	22.30	8.52	21.76	8.57
T_6 Diethyl sulphate@2500ppm	21.86	8.48	6.72	21.36	8.39	21.68	8.11
T_7 Diethyl sulphate@3500ppm	23.58	8.87	7.25	21.94	8.38	23.07	8.34
T_8 Diethyl sulphate@4500ppm	22.13	7.58	6.50	20.01	6.92	18.90	7.18
T_9 Control	23.40	8.38	7.08	21.29	8.13	21.32	7.70
SEm±	0.32	0.24	0.15	0.40	0.25	0.81	0.29
$CD_{0.05}$	0.97	0.73	0.44	1.21	0.76	2.43	0.87

3.5 Leaf Length (cm)

In (Table 1) T_1 i.e., @1500ppm Sodium Azide (9.48cm) the maximum leaf length of calendula cv. calypso orange was observed. At low concentration of sodium azide, expression of related genes and the molecular level that affects the gene or groups of genes and these genes could be those controlling the synthesis of growth regulators such as auxins and cytokinins and same result mentioned by Cain et al*.* 2006 and Joshi et al*.* [10]. The smallest leaf length of calendula cv. calypso orange per plant was observed in T_8 i.e., Diethyl Sulphate@ 4500ppm (6.92cm), which was statistically inferior due to its cell elongation processes are inhibited by high concentrations of diethyl sulphate, which limits growth and prevents plants from developing their leaves to their normal length and these findings as reported by Sharma et al*.* (2023).

3.6 Plant Spread (cm^2)

The maximum plant spread data was observed (Table 1) in T_1 i.e., @1500 ppm Sodium Azide (24.89 cm^2) which was statistically at par with T_7 i.e., Diethyl Sulphate@ 3500ppm (23.07cm^2). The stimulatory effect of low dose sodium azide may contribute to better nutrient absorption and hormonal signalling as reported by Kayalvizhi et al*.*, 2023. The lowest data of plant spread was noted in T_8 i.e., Diethyl Sulphate@ 4500ppm (18.90cm^2) which was statistically at par with T_2 i.e., Sodium Azide @2500ppm (21.02cm^2) and T_9 i.e., control (21.32cm^2) the study demonstrated the toxicity of diethyl sulphate at high concentrations, which can cause physiological disturbances in plant cells and metabolic processes. This study was reported by Sharma et al*.* (2023).

3.7 Diameter of Main Stem (mm)

There was significant effect of chemical mutagens on calendula cv. calypso orange in the Table 1. The main stem's maximum diameter was recorded in T_1 1500ppm Sodium Azide (9.39mm), which was statistically at par with T_5 i.e., Diethyl Sulphate@ 1500ppm. (8.57mm). T_8 Diethyl Sulphate@4500ppm (7.18mm). Which might be due to the stimulatory effect of the mutagen may be linked to an increase in the rate of cell division or elongation as well as an activation of auxin (Zaka et al*.,* 2004), [10]. The minimum diameter of main stem was noted in T_8 Diethyl Sulphate@4500ppm (7.18mm) which was statistically at par with T_2 i.e., Sodium Azide @ 2500ppm (7.52mm) T_4 i.e., Sodium Azide @ 4500ppm (7.97mm) T_9 i.e., control (7.70mm), diethyl sulphate is the highly toxic compound at higher concentration that can lead to cell death, inhibition of cell division, and disruption of vascular tissues in the main stem. These findings are related with Kannan et al. [16].

4. CONCLUSION

From the present study it can be concluded that the lowest concentration of Sodium Azide (1500ppm) reported the best performance of vegetative parameters such as plant height, number of leaves per stem, number of stems per plant, stem length, leaf length, plant spread, and diameter of main stem.

From the results, it can be concluded that Sodium Azide @1500ppm is best dosage for *Calendula officinalis* cv. Calypso orange.

Using chemical mutagen like Sodium azide to induce mutations have various future prospects that are used for produced the different colour of flowers. This genetic diversity can potentially result in unique traits, such as altered leaf shapes, sizes or branching patterns in calendula flowers.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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