

Research Article

## Effect of chemical mutagens (Ethyl methane sulphonate and colchicine) on African marigold (*Tagetes erecta*)

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### Article Info

<https://doi.org/10.31018/jans.v17i2.6167>

Received: September 4, 2024

Revised: May 19, 2025

Accepted: May 25, 2025

### How to Cite

Pathania, S. and Sahare, H. (2025). Effect of chemical mutagens (Ethyl methane sulphonate and colchicine) on African marigold (*Tagetes erecta*). *Journal of Applied and Natural Science*, 17(2), 697 - 701. <https://doi.org/10.31018/jans.v17i2.6167>

### Abstract

*Tagetes erecta*, commonly known as African marigold, is an annual or perennial herbaceous plant that belongs to the family Asteraceae. There is a need for mutation breeding, which allows for the development of novel cultivars that could be more valuable and highly demanding in the market. The present study aimed to find the effect of chemical mutagens on the growth and flowering of marigold cv. African marigold. The trial was statistically laid out in RBD with 3 replications and 3 replications. The treatment comprised of EMS (0.2%, 0.4%, 0.6%), (EMS 0.2% + Distilled Water 100ml), Colchicine (0.001%, 0.002%, 0.005%), (Colchicine 0.001% + Distilled Water 100ml), (EMS 0.2% + Colchicine 0.005%) and (EMS 0.6% + Colchicine 0.001%) and control. Results revealed that among all the chemical mutagens, maximum changes were in vegetative parameters like plant height, plant spread, number of leaflets, stem diameter and number of branches for the treatment T<sub>6</sub> (Colchicine 0.002%) and T<sub>8</sub> (Colchicine 0.001% + Distilled Water 100ml) and in flowering parameters like flower yield. The maximum changes were recorded in treatment T<sub>5</sub> (Colchicine 0.001%). It can be concluded that chemical mutagens can enhance a plant's vegetative growth and flowering, leading to the sustainable production of marigold. The study also revealed that colchicine and EMS might affect plant development characteristics and showed positive results in enhancing marigold's vegetative and floral parameters.

**Keywords:** Colchicine, Enhancement, Ethyl methane sulphonate (EMS), Growth, Marigold, Mutation, Mutagenesis

## INTRODUCTION

Marigolds, are widely cultivated ornamental flowering plants in India, often used as bedding or pot plants which are native to Mexico and Central America. The African marigold (*Tagetes erecta*) is a notable species, reaching 20 to 90 cm in height and characterized by its vibrant yellow to red flowers (Gupta *et al.*, 2022). India is a major producer, contributing 75-80% of the global marigold output, with Andhra Pradesh, Uttar Pradesh, and Tamil Nadu as leading states. Mutation plays a crucial role in evolution, and mutagenesis has become significant in biological sciences, particularly for enhancing the genetic base of germplasm in plant breeding. Induced mutagenesis has successfully improved various ornamental plants, including chrysanthemums and gerberas, by altering their flower and leaf characteristics and physiological traits (Wu *et al.*, 2011). In-

duced mutations can create new plant varieties with desirable traits, such as improved yield, disease resistance, or stress tolerance. Mutagenesis increases genetic variability within a species, which is essential for breeding programs to select specific traits (Susrama and Yuliadhi, 2020). Mutagenesis can enhance quality characteristics like flavour, colour, and nutritional content in crops, making them more appealing to consumers. It can be used to modify flowering time and maturity, allowing for better synchronization with environmental conditions or market demands (Fahmy *et al.*, 2020). Many mutant varieties exist, but their current use in agriculture and horticulture is uncertain, as they are often not labelled as mutagenic. Researchers have explored chemical mutagens due to the adverse effects of ionizing radiation (Singh, 2019). Common chemical mutagens include alkylating agents like colchicine and ethyl methane sulphonate (EMS), which are favoured

for their effectiveness and ease of disposal. Chemical mutagens like colchicine are often used to induce polyploidy, leading to increased size and vigour in plants (Patil *et al.*, 2017). EMS and colchicine are crucial tools in plant mutation breeding. EMS is primarily used for inducing point mutations, enhancing genetic diversity, and improving traits in various crops (Lenawaty *et al.*, 2022). Together, they play significant roles in developing new plant varieties with desirable characteristics, contributing to agricultural innovation and sustainability (Aravind and Dhanavel, 2022). The research will evaluate the impact of these chemical mutagens on the vegetative and flowering growth of marigolds in the M1 and M2 generations, aiming to create new genetic variability for breeding purposes. This study aimed to investigate the effects of different concentrations of colchicine and EMS on the morphological characteristics of African marigolds and the potential for inducing mutations.

## MATERIALS AND METHODS

The current research was conducted at the Research Farm, Department of Horticulture, College of Agriculture, Lovely Professional University, Punjab, India, in 2023-2024. Seedlings of African marigold (*Tegetas erecta*) were treated with different concentrations of EMS and colchicine for 24 hrs and then planted in the field area. The seedlings were treated with different concentrations of chemical mutagens *i.e.* EMS (0.2%, 0.4%, 0.6%), (EMS 0.2% + Distilled Water 100ml), colchicine (0.001%, 0.002%, 0.005%), (Colchicine 0.001% + Distilled Water 100ml), (EMS 0.2% + colchicine 0.005%) and (EMS 0.6% + colchicine 0.001%) by dipping in the chemical solutions for 24 hours. In the control, normal water was used to dip the seedlings. The treated seedlings with untreated control were planted in the field. During the research period, all standard cultural practices were followed. Measurements for plant height, plant spread, number of leaflets, number of branches, stem diameter, and flower yield were taken at 21-day intervals until reaching 75 days.

### Statistical analysis

All investigated qualities were analysed using One-way analysis of variance (ANOVA). Furthermore, least significant difference (LSD) values at the 5% probability level were calculated.

## RESULTS AND DISCUSSION

The present research demonstrated that EMS and colchicine influenced plant development characteristics. The results indicated that after 21 days after planting (DAP), the maximum plant height (8.37 cm) was recorded in treatment T<sub>6</sub> (colchicine 0.002%), while the minimum height (5.33 cm) was observed in treatment T<sub>3</sub>

(EMS 0.6%). After 42 DAP, the maximum plant height (21.50 cm) was noted in treatment T<sub>1</sub> (EMS 0.2%), whereas the minimum height (17.17 cm) was recorded in treatment T<sub>6</sub> (colchicine 0.002%). After 63 DAP, the maximum plant height (43.58 cm) was again found in treatment T<sub>1</sub> (EMS 0.2%), while the minimum height (37.08 cm) was in treatment T<sub>2</sub> (EMS 0.4%) (Table 1). These findings suggest that the application of a specific concentration of EMS effectively increased plant height. This enhancement in plant characteristics is likely due to increased enzyme activity that promotes the biosynthesis of plant hormones such as auxin and cytokinin, leading to overall growth improvements. The stimulation of cell growth through hormone biosynthesis contributes to various enhancements, including an increased plant height. Hence, using the specific concentrations of EMS can increase plant height as relatively similar effects were reported by Yoosumran, V., *et al.* (2018) in chrysanthemum.

The results indicated that after 21 days (DAP), the maximum plant spread (16.68 cm) was recorded in treatment T<sub>6</sub> (colchicine 0.002%), while the minimum plant spread (11.83 cm) was observed in treatment T<sub>11</sub> (Control). After 42 days (DAP), the maximum plant spread (21.83 cm) was noted in treatment T<sub>1</sub> (EMS 0.2%), whereas the minimum (14.92 cm) was again in treatment T<sub>11</sub> (Control). After 63 days (DAP), treatment T<sub>6</sub> (colchicine 0.002%) recorded the maximum plant spread (43.92 cm), while the minimum (32.00 cm) was found in treatment T<sub>2</sub> (EMS 0.4%) (Table 1). These findings suggest that the application of colchicine effectively enhanced plant spread, as it induces polyploidy and chromosome doubling by preventing the formation of microtubules, which can improve overall plant spread and a relatively similar effect was recorded by Wu., *et al.* (2011) in Chinese bellflower.

The results indicated that in the case of leaflets, after 21 days (DAP), the maximum number (9.00) was recorded in treatment T<sub>6</sub> (colchicine 0.002%), while the minimum count was observed in treatment T<sub>3</sub> (EMS 0.6%, 4.58). After 42 days (DAP), treatment T<sub>6</sub> (colchicine 0.002%) again showed the maximum number of leaflets (24.83), whereas the minimum was recorded in treatment T<sub>11</sub> (Control, 15.67). After 63 days (DAP), the maximum number of leaflets (49.83) was again noted in treatment T<sub>6</sub> (colchicine 0.002%), while the minimum number of leaflets (33.75) was found in treatment T<sub>11</sub> (Control) (Table 1). These findings suggest that the application of colchicine effectively increased the number of leaflets, as colchicine is known to induce polyploidy in plants by disrupting the normal process of mitosis. This disruption leads to chromosome doubling, which can result in various morphological changes, including a larger number of leaflets and a relatively similar effect was recorded by Aravind and Dhanavel (2022) and Dewi (2018) in marigold spp *Ta-*

**Table 1.** Effect of ethyl methane sulphonate and colchicine on plant height, plant spread and number of leaflets of marigold at 21DAP, 42DAP and 63DAP

Treatments	Plant height (cm)			Plant spread (cm)			Leaflets (No.)		
	21 DAP	42 DAP	63 DAP	21 DAP	42 DAP	63 DAP	21 DAP	42 DAP	63 DAP
T <sub>1</sub> Ethyl Methane Sulfonate (0.2%)	5.92	21.50	43.58	13.50	21.83	39.33	5.08	23.42	45.83
T <sub>2</sub> Ethyl Methane Sulfonate (0.4%)	5.38	19.25	37.08	13.47	19.00	32.00	5.00	16.17	34.50
T <sub>3</sub> Ethyl Methane Sulfonate (0.6%)	5.33	19.17	41.50	13.70	20.08	40.25	4.58	21.83	45.00
T <sub>4</sub> Ethyl Methane Sulfonate (0.2%) + Distilled Water (100ml)	6.91	20.58	38.50	14.85	21.42	35.67	6.75	23.42	47.17
T <sub>5</sub> Colchicine (0.001%)	7.89	19.00	39.42	15.52	21.17	40.17	7.42	24.08	45.50
T <sub>6</sub> Colchicine (0.002%)	8.37	17.17	41.75	16.68	20.25	43.92	9.00	24.83	49.83
T <sub>7</sub> Colchicine (0.003%)	8.08	16.08	43.50	13.62	21.67	41.33	7.75	18.42	36.17
T <sub>8</sub> Colchicine (0.001%) + Distilled Water (100ml)	7.58	21.17	41.92	15.17	20.00	34.83	6.75	20.92	42.42
T <sub>9</sub> Ethyl Methane Sulfonate (0.2%) + Colchicine (0.005%)	7.29	17.83	42.50	14.17	18.83	35.58	7.83	18.17	37.58
T <sub>10</sub> Ethyl Methane Sulfonate (0.6%) + Colchicine (0.001%)	8.17	17.58	41.25	14.83	19.58	37.95	8.83	23.00	43.83
T <sub>11</sub> Control	6.33	18.92	42.17	11.83	14.92	34.92	6.25	15.67	33.75
Sem	0.93	2.19	3.38	0.88	1.76	3.58	1.10	3.93	7.21
CD 5%	2.75	6.47	9.98	2.58	5.19	10.57	3.24	11.60	21.25
CD 1%	3.75	8.82	13.61	3.52	7.09	14.41	4.41	15.82	28.99
CV	23.00	20.06	14.22	10.60	15.34	16.41	27.77	32.58	29.74

DAP: Days after planting

*getes erecta*.

The results revealed that after 42 days (DAP), the maximum stem diameter (6.44 mm) was recorded in treatment T<sub>8</sub> (Colchicine 0.001% + Distilled Water 100 ml), while the minimum diameter (1.52 mm) was observed in treatment T<sub>3</sub> (EMS 0.6%). After 63 days, treatment T<sub>8</sub> (Colchicine 0.001% + Distilled Water 100 ml) again showed the maximum stem diameter (7.03 mm), whereas treatment T<sub>3</sub> (EMS 0.6%) recorded the minimum (2.13 mm) (Table 2). These findings suggest that the application of colchicine effectively enhanced stem diameter, likely due to its role in inducing polyploidy and chromosome doubling, which can improve various plant traits and a relatively similar effect was recorded by Din A. Qadri *et al.* (2023) in chrysanthemum and Karuppaiah *et al.*, (2004) in pot marigold.

In the case of branches, after 42 days (DAP), the maximum number of branches (13.50) was observed in treatment T<sub>6</sub> (colchicine 0.002%), while the minimum number (9.33) was found in treatment T<sub>2</sub> (EMS 0.4%)

while after 63 days (DAP), treatment T<sub>6</sub> (colchicine 0.002%) again recorded the maximum number of branches (24.83), whereas treatment T<sub>2</sub> (EMS 0.4%) showed the minimum (19.58) number of branches (Table 2). These findings suggest that applying colchicine effectively increased the number of branches, likely due to its role in inducing polyploidy and chromosome doubling, which can enhance plant characteristics and a relatively similar effect was recorded by Dewi (2018) and Kumari (2024) and (Sajjad *et al.* 2013) in marigold.

In the case of the flower yield, after 75 days of planting, the maximum flower yield (40.00) was observed in treatment T<sub>5</sub> (colchicine 0.001%), while the minimum yield (31.33) was recorded in treatment T<sub>11</sub> control. This suggests that colchicine application effectively boosts flower yield, likely due to flower size, quantity, and overall plant health enhancements as a relatively similar effect was recorded by (Jadagoudar. 2019) and (Patil *et al.*, 2017) in marigolds (Table 2).

**Table 2.** Effect of ethyl methane sulphonate and colchicine on stem diameter, number of branches and flower yield of marigold on 42DAP, 63DAP and 75DAP

Treatments	Stem diameter (mm)		Branches (No.)		Flower yield (No.)
	42 DAP	63 DAP	42 DAP	63 DAP	75 DAP
T <sub>1</sub> Ethyl Methane Sulfonate (0.2%)	2.48	3.05	11.25	21.50	36.00
T <sub>2</sub> Ethyl Methane Sulfonate (0.4%)	3.41	3.97	9.33	19.58	34.00
T <sub>3</sub> Ethyl Methane Sulfonate (0.6%)	1.52	2.13	9.92	20.42	35.67
T <sub>4</sub> Ethyl Methane Sulfonate (0.2% ) + Distilled Water (100ml)	1.61	2.27	10.08	20.50	36.00
T <sub>5</sub> Colchicine (0.001%)	2.67	3.32	12.50	22.58	40.00
T <sub>6</sub> Colchicine (0.002%)	2.60	3.14	13.50	24.83	37.00
T <sub>7</sub> Colchicine (0.003%)	1.69	2.36	11.17	22.83	36.00
T <sub>8</sub> Colchicine (0.001%) + Distilled Water (100ml)	6.44	7.03	12.08	21.42	38.00
T <sub>9</sub> Ethyl Methane Sulfonate (0.2%) + Colchicine (0.005%)	1.89	2.51	11.00	22.08	37.00
T <sub>10</sub> Ethyl Methane Sulfonate (0.6%) + Colchicine (0.001%)	2.05	3.15	12.92	23.25	35.00
T <sub>11</sub> Control	1.87	2.54	11.42	21.75	31.33
Sem	0.20	0.17	0.90	1.07	0.51
CD 5%	0.58	0.50	2.67	3.17	1.50
CD 1%	0.79	0.68	3.64	4.32	2.05
CV	13.19	9.12	13.75	8.51	2.45

DAP: Days after planting

## Conclusion

The current study determined that specific concentrations of EMS led to improved traits across various parameters; however, colchicine consistently yielded superior results in promoting the growth as well as the vegetative and floral characteristics of marigolds. Among all the concentrations of chemical mutagens, the most significant changes in morphological parameters-such as plant height, plant spread, number of leaflets, stem diameter, and number of branches were observed in treatment T<sub>6</sub> (colchicine at 0.002%) and T<sub>8</sub> (colchicine + DW at 0.001% + 100 mL). Regarding flowering parameters, treatment T<sub>5</sub> (colchicine at 0.001%) demonstrated favorable outcomes in flower yield. As comparing this research with previous works where mostly EMS showed mutagenic variability, it shows that specific concentrations of colchicine used can affect the plant's morphological characters by development of polyploidy level, which leads to chromosome doubling and can produce many mutagenetic effects on plant. Therefore, it can be concluded that the application of colchicine effectively enhanced plant

characteristics, indicating the necessity for further studies to achieve more comprehensive mutations.

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