

Impact of physical and chemical mutagens on vegetative growth of China aster [*Callistephus chinensis* (L.) Nees] cv. Phule Ganesh Pink

ABSTRACT

The present study entitled “Impact of physical and chemical mutagens on vegetative growth of China aster [*Callistephus chinensis* (L.) Nees] cv. Phule Ganesh Pink” was carried out in two different experiments of mutation during September, 2021 to March, 2022 at Floriculture Research Farm, ASPEE College of Horticulture, Navsari Agricultural University, Navsari, Gujarat. This experiment study comprised was conducted for two separate mutation experiments carried out over two generations, and laid out using Randomized Block Design (RBD) with three and five replications. During 2021-22, seedlings of China aster var. Phule Ganesh Pink were treated exposed to with different doses of various mutagens treatments, which included four treatments levels of gamma rays (0.25 kR, 0.50 kR, 0.75 kR and 1.00 kR) and another four treatments levels of chemical mutagens (0.15 % DES, 0.20 % DES, 0.20 % EMS and 0.25 % EMS) along with a control group and without mutagenic treatment as control. The effects of different doses of chemical mutagen on vegetative aspects were thoroughly investigated and. Fifteen mutants selected from the M₁ generation. Generally, lower doses of chemical mutagens *i.e.* 0.15 % DES showed positive effect on growth related attributes as compared to higher doses. Significantly, the maximum survival per cent *i.e.*, 95.08 was observed in T₉ (Control) while, the maximum plant height (55.95 cm) and number of branches (9.60) were recorded in T₅ (0.15 %). The highest number of variations were obtained in treatments of chemical mutagens in which maximum number of mutants were noted in T₆ (0.20 % EMS).

Keywords: China aster, Mutation, Mutagens, Vegetative attributes and Flowering parameters.

INTRODUCTION

China aster [*Callistephus chinensis* (L.) Nees] is one of the commercially grown crops which is half hardy winter flowering annual crop. It is diploid plant with chromosome number $2n=18$ and belongs to family Asteraceae. It is primarily originated from Northern China (Desai, 1967). The evolution of China aster [*Callistephus chinensis* (L.) Nees] has a history of remarkable variations. According to Emsweller *et al.* (1937), the original plant had single flowers with two or more rows of blue, violet or white ray florets. The stature was medium tall and height of the plant ranged from 18 to 24 inches. The first change in the flower had been the prolongation or development of central florets and the production of quilled flowers. Germans developed double forms in aster during 18th century and hence, it is also called as German asters. Advancement of the aster evolution and large-scale seed production by Germans led to the introduction of branching types like, tall, medium tall and dwarf types and this contributed to evolutionary improvement in China aster.

China aster is an important annual crop of our country and grown throughout the world due to existing of various vibrant colours ranging from violet, purple, magenta, pink

and white; forms, sizes and pretty good post-harvest life (Diltaet *et al.*, 2007). It is grown commercially as cut flower for flower arrangement, interior decoration and loose flower for garland making, worshipping, *etc.* (Munikrishnappa, 2013). It can also be grown as bedding plant and potted plant in landscaping as well it is a richest source of natural pigments (Bhargav *et al.*, 2016). In India, China aster is commercially grown by marginal and small farmers of Karnataka, Tamil Nadu, Telangana, Andhra Pradesh, Maharashtra and West Bengal states (Kumari *et al.*, 2017). There is a need to develop novel flower colours and forms in China aster as the consumer preferences changes frequently. Although, China aster is being grown in considerable areas, its cultivation is concentrated around big cities and there is a need to popularize it under different agro climatic conditions.

Mutation is a sudden heritable change in a characteristic of an organism and treating a biological material with a mutagen in order to induce mutations is known as mutagenesis. When mutations are induced for crop improvement, the entire operation of the induction and isolation *etc.* of mutants is termed as mutation breeding (Singh, 2016). Mutation occur constantly in nature and these spontaneous mutations are the natural genetic variation that promote the development of new varieties of enhancement of the prevailing ones. The decelerating natural mutation rate of 1×10^{-8} to 10^{-9} per generation in molecular organisms forces to choose induced mutation through physical and chemical agents (Mba, 2012). In past 80 years, physical mutagen, mostly ionizing radiations, have been used widely for inducing hereditary aberrations and more than 70 % of mutant varieties were developed using physical mutagenesis (Mba *et al.*, 2013).

Gamma radiation from radioactive cobalt-60 (^{60}Co) is widely used. It has shorter ~~wave length~~wavelength and therefore, possess more energy than protons and X-rays which gives them ability to penetrate deeper into the tissue (Amano, 2006). The effect of chemical mutagens on plant materials is generally considered milder. An advantage of chemical mutagenic agents is that they can be applied without complicated equipment or facilities. The ration of mutational to undesirable modifications is generally higher for chemical mutagens than for physical mutagens. A clear advantage of the point mutations created by chemical mutagen is their potential to generate not only loss of function but also gain of function phenotypes if the mutation leads to a modified protein activity or affinity (Acquaah, 2006).

Materials and Methods

The present study entitled “Impact of physical and chemical mutagens on vegetative growth of China aster [*Callistephus chinensis* (L.) Nees] cv. Phule Ganesh Pink” was carried out in two different experiments of mutation during September, 2021 to March, 2022 at Floriculture Research Farm, ASPEE College of Horticulture, Navsari Agricultural University, Navsari, Gujarat. The experiment was conducted for two generations and laid out in Randomized Block Design (RBD) with three and five replications. During 2021-22, seedlings of China aster var. Phule Ganesh Pink were treated with different doses of various mutagens which included four treatments of gamma rays (0.25 kR, 0.50 kR, 0.75 kR and 1.00 kR) and another four treatments of chemical mutagen (0.15 % DES, 0.20 % DES, 0.20 % EMS and 0.25 % EMS) and without mutagenic treatment as control.

For physical mutagenic treatments, the seedlings were irradiated with gamma rays from Bhabha Atomic Research Center, Trombay, Maharashtra with different doses of 0.25 kR, 0.50 kR, 0.75 kR and 1.00 kR on 2nd November, 2021. Diethyl sulphate and Ethyl methyl sulphate were used for chemical mutagenic treatments at different doses. Chemical solution was prepared by diluting 125 µl of chemical mutagens in 100 ml of water and then added more water to make solution of 250 ml of 0.15 %. Likewise, 500 µl and 625 µl of DES as well as EMS were diluted in 100 ml of water and then added remaining water to make it upto for making solutions of 250 ml of 0.15 %, 0.20 %, 0.20 % and 0.25 %, respectively. Forty-eight seedlings were selected per treatment per replication and were treated with different concentrations of chemical mutagens by immersing their roots in the chemical solutions of 250 ml each for 3 hours. After the treatments, these seedlings were removed from the chemical solutions and washed in running tap water for 20 minutes to remove the chemical mutagens adhering to roots of the treated seedlings. The healthy seeds of China aster var. Phule Ganesh Pink were procured from Floriculture Research Farm, ASPEE College of Horticulture, Navsari Agricultural University, Navsari. Seedlings were kept in normal water to keep untreated for control.

The standard method of analysis of variance technique appropriate to the Randomized Block Design (RBD) as described by Panse and Sukhatme (1985) was used. The data were analyzed with the technical help received from computer centre, ASPEE College of Horticulture, N.A.U., Navsari. The treatment differences were tested by employing 'F' test at five per cent level of significance on the basis of null hypothesis. The appropriate standard errors (S.Em. ±) were calculated in each case and the Critical Difference (CD) at five per cent level of probability was worked out, where the treatment effects were found significant under 'F' test. The percentage co-efficient of variation (CV %) was also worked out for all the cases.

Result and Discussion

1. Survival percentage (%)

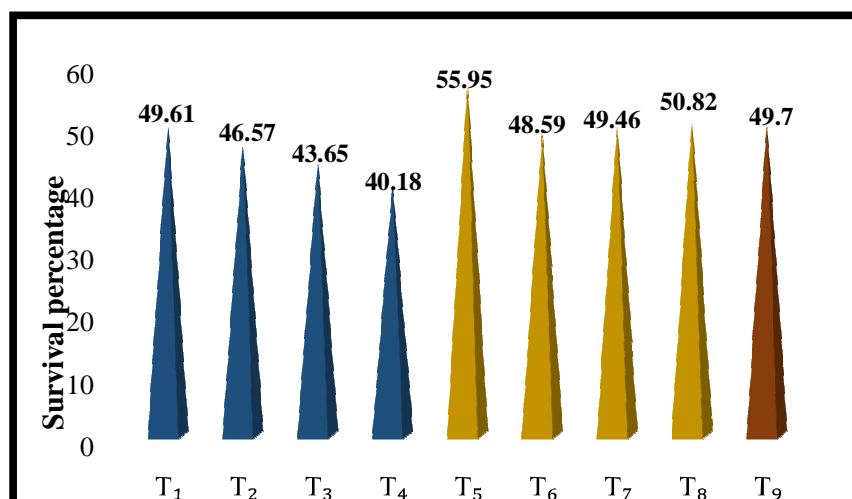
~~The data on plant pertaining to survival percentage of plants~~ at thirty days after transplanting are presented in Table 1 and graphically depicted in Fig. 1. ~~Analysis-inquisition~~ of the data ~~showedrevealed~~ that ~~the~~ highest survival per cent ~~i.e.(95.08)~~ was observed in T₉ (Control), which was ~~statistically similar tofound at par with~~ T₇ (93.87 %), T₅ (93.45 %) and T₈ (86.39 %). ~~In contrast, -whereas,~~ T₄ (1.00 kR) recorded minimum survival (60.96 %). ~~Further, -it~~ was ~~clear-evident~~ from the data that ~~the~~ reduction in per cent survival was increased with increase in the concentrations of physical and chemical mutagenic treatment. However, seedling survival per cent in treatments of gamma rays is lower than in chemical mutagens, with the exception that concentrations of DES (0.20%) demonstrated lower survival than treatment of 0.50 kR.

The ~~response of the~~ survival percentage ~~to the dose displayed~~ exhibited a negative relationship ~~with increasing mutagen doses~~, indicating that ~~the increased~~ higher dose of mutagen ~~decreased ceases~~ the survival rate. The ~~concept of~~ LD₅₀, ~~in particular, is predicted~~ suggests ~~that on the premise that they produce limited genome effects at~~ lower doses of mutagens ~~caused limited genomic effects~~ that induce phenotypical alterations, ~~but while~~ higher dose may ~~produce~~ result in substantial genome effects that frequently lead to aberrations or abnormalities. Survival was reduced following gamma rays exposure due to inactivation and/or decrease in auxin content, which impacts cell division and ultimately ~~results in~~ leads to poor establishment and survival (Gordon, 1957 and Mahureet *et al.*, 2010). ~~Additionally, the or deadly lethal~~ effects of gamma rays ~~produced by~~ are linked to chromosomal abnormality (Datta and Benerji, 1993). These results are ~~parallel consistent with~~ the ~~to~~ findings of Banerji and Datta (2005), Kim *et al.* (2016), Patel *et al.* (2018) in gladiolus and Din *et al.* (2020) in chrysanthemum.

In ~~the case of~~ chemical mutagenic treatment, the ~~drop decline~~ in plant survival may be attributed to the ~~ereation~~ formation of ~~some~~ toxic compounds ~~by through~~ specific biochemical process, ~~which causes leading to~~ cell death, ~~resulting in and~~ plant mortality (D'Amato and Ostenhof, 1956; Gordon, 1956). The ~~higher~~ reduction in survival ~~rate observed with was high~~ in gamma radiation, ~~as compared to chemical treatments, can be explained by the fact and this is evident~~ that chemicals ~~typically cause~~ produce only point mutation, whereas, radiations ~~normally cause usually result in~~ chromosomal rearrangement and deletions (Bhat *et al.*, 2007). ~~Moreover~~ Furthermore, DES break ~~definite specific~~ chromosomes of the genome and sometimes also break definite region of a chromosome, which induce more chromosomal fragments and fewer chromosome recombination (Gaul, 1970) which ultimately kill the growing portion and lead mortality. This finding is in conformity with Ghani *et al.* (2013) in barberton daisy, Arvind and Dhanavel (2022) in marigold and Chandana (2021) in China aster.

Table 1: Effect of different mutagens on plant height of China aster var. Phule Ganesh Pink

Treatment	Survival percentage
T ₁ : 0.25 kR gamma rays	68.08
T ₂ : 0.50 kR gamma rays	84.23
T ₃ : 0.75 kR gamma rays	73.81
T ₄ : 1.00 kR gamma rays	60.96
T ₅ : DES @ 0.15 %	93.45
T ₆ : DES @ 0.20 %	82.88



T ₇ : EMS @ 0.20 %	93.87
T ₈ : EMS @ 0.25 %	86.39
T ₉ : Control	95.08
SEm _±	3.18
CD at 5 %	9.20
CV %	8.66

Fig. 1: Effect of different mutagens on survival percentage of Chinaaster var. Phule Ganesh Pink

B. Plant height (cm)

Plant height of China aster var. Phule Ganesh Pink was affected by various mutagens that have been summarized in Table 2 and graphically presented in Figure 2.

An appraisal examination of the data showed that, among different mutagenic treatments, significantly maximum plant height (55.95 cm) was recorded in T₅ (DES @ 0.15 %) which was at par with T₈ (0.25 % EMS), T₉ (Control), T₁ (0.25 kR), T₇ (0.20 EMS) and T₆ (0.20 % EMS) *i.e.* 50.82 cm, 49.70 cm, 49.61 cm, 49.46 cm and 48.59 cm, respectively.

While, plant remained dwarf (40.18 cm) in T₄ (1.00 kR). Plant height was positively increased with the increased doses of EMS whereas, in case of DES mutagenic treatments, plant height was started declining with higher dose. In case of gamma ray treatments, plant height decreased with increased doses. It was apparent from the data presented that chemical mutagens have an increased effect than gamma rays on enhancing plant height.

Plant height is a quantitative trait ~~which is~~ predominantly controlled by polygenes and each gene contributes a small effect which externally expressed in plant morphology. Moreover, increases in the plant height may be due to the reason that certain chemical mutagens produce single base substitution with different mutation spectra, because of which broad variation occur in morphological parameters as compared to control (Abdullah *et al.*, 2009). The stimulatory effect of the mutagen may be attributed to the increase in the rate of cell division or cell elongation as well as an activation of auxin as reported by (Zaka *et al.*, 2004 and Joshi *et al.*, 2011). This is in line with the findings of Banerji and Datta (2005) and Kim *et al.* (2016) in chrysanthemum.

Reduction in vegetative characters by gamma rays treated plants depends on the nature and degree of chromosomal injury or morphological, cytological and physiological disturbance induced by irradiation and the decline in interior auxin manufacture, leading to plummeting growth of the plant (Banerji and Datta, 2002). Also, it may be due to the inactivation of auxin synthesis, nature and degree of chromosomal aberration (Singh *et al.*, 2015). These results are parallel to findings of Purentee *et al.* (2020) and Nasri *et al.* (2021) in chrysanthemum.

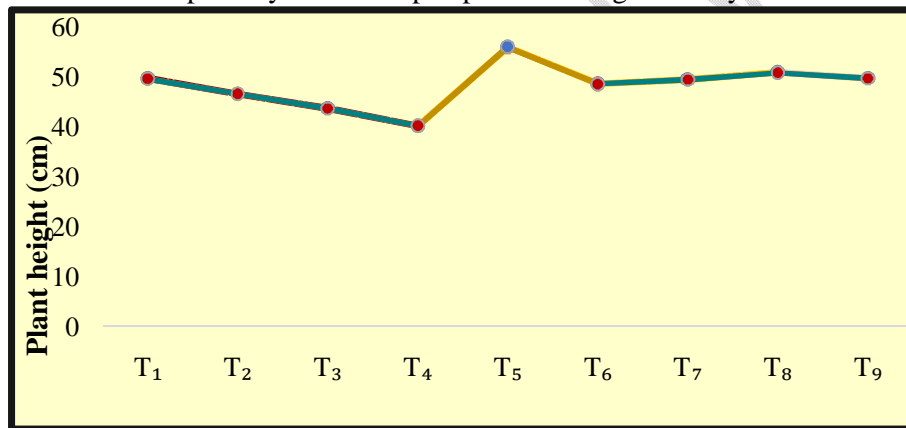
Table 2: Effect of different mutagens on plant height of Chinaaster var. Phule Ganesh Pink

Treatment	Plant height (cm)
T ₁ : 0.25 kR gamma rays	49.61
T ₂ : 0.50 kR gamma rays	46.57
T ₃ : 0.75 kR gamma rays	43.65
T ₄ : 1.00 kR gamma rays	40.18
T ₅ : DES @ 0.15 %	55.95
T ₆ : DES @ 0.20 %	48.59
T ₇ : EMS @ 0.20 %	49.46
T ₈ : EMS @ 0.25 %	50.82
T ₉ : Control	49.70
SEm_±	2.85
CD at 5 %	8.25
CV %	13.20

Fig. 2: Effect of different mutagens on plant height (cm) of Chinaaster var. Phule Ganesh Pink

C. Number of Primary Branches

The number of primary branches per plant was significantly affected due to various



mutagenic treatments and the results of the same are presented in Table 3 and explained graphically in Figure 3.

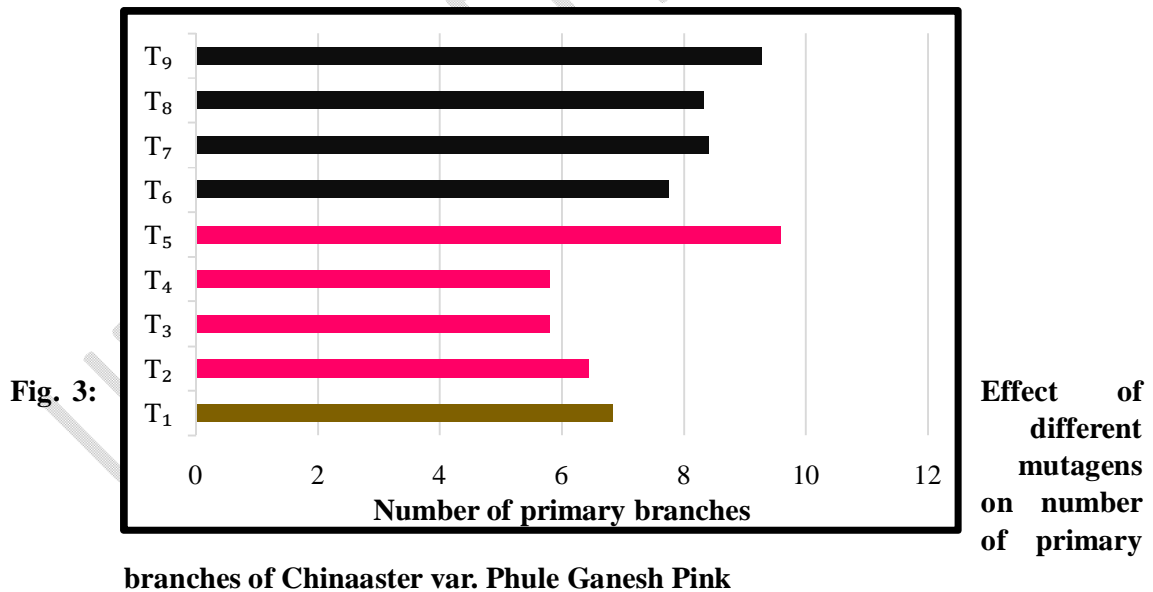
Data revealed that the maximum number of primary branches (9.60) were found in T₅ (0.15 % DES) which was at par with treatment T₉ (Control), T₇ (0.20 % EMS) and T₈ (0.25 % EMS) i.e. 9.28, 8.40 and 8.32, respectively in China aster var. Phule Ganesh Pink. The least number of primary branches per plant (5.80) were observed in 0.75 kR and 1.00 kR. (T₃ and T₄). The number of primary branches per plant were decreasing with increasing doses of gamma rays, DES and EMS.

The increase in the production of branches implies a positive effect on the better framework and flower production of the plants. The physiological effects of DES and their hydrolysis products could also be the reason for increasing the number of branches. The lower and intermediary doses or concentrations of gamma rays, DES and EMS have a stimulatory effect on cell replication and elongation, yielding a biopositive vegetative effect in comparison to higher ones (Chandrashekar, 2014). Decrease in primary branches in treatments of gamma rays is a result of proliferative capacity of cell. Above findings are in

conformity with EI-Nashar and Asrar (2016) in calendula, Patel *et al.* (2018) in gladiolus and Chandana (2021) in China aster.

Table 3: Effect of different chemical mutagens on number of primary branches of Chinaaster var. Phule Ganesh Pink

Treatment	Number of primary branches
T ₁ : 0.25 kR gamma rays	6.84
T ₂ : 0.50 kR gamma rays	6.44
T ₃ : 0.75 kR gamma rays	5.80
T ₄ : 1.00 kR gamma rays	5.80
T ₅ : DES @ 0.15 %	9.60
T ₆ : DES @ 0.20 %	7.76
T ₇ : EMS @ 0.20 %	8.40
T ₈ : EMS @ 0.25 %	8.32
T ₉ : Control	9.28
SEm±	0.49
CD at 5 %	1.43
CV %	14.57



Selection of Putative Mutants for M₁ Generation

It's important to note that the specific criteria for selecting mutants in flower crops may vary depending on the type of flower crop, market demands, consumer preferences, and regional or cultural factors. The selection process often involves a combination of visual assessments and trait measurements to identify mutants with the desired traits. In the M₁ generation, a total of 4 mutants were found (Table 4 and Photo 1) displayed variations in plant type and number of branches, respectively.

Table 4: Salient features of selected mutants of China aster for M₁ generations

Mutagenic treatments	Salient features	Genotypes
T ₄ (1.00 kR)	Spreading, maximum number of flowers with small diameter, suitable for landscaping	ASTM 3
T ₈ (0.20% EMS)	Erect, floriferous, deep purplish pink coloured flower	ASTM 5
T ₅ (0.15% EMS)	Spreading and profuse flowering	ASTM 7
T ₆ (0.20% DES)	Erect growing habit with red coloured flower	ASTM 9

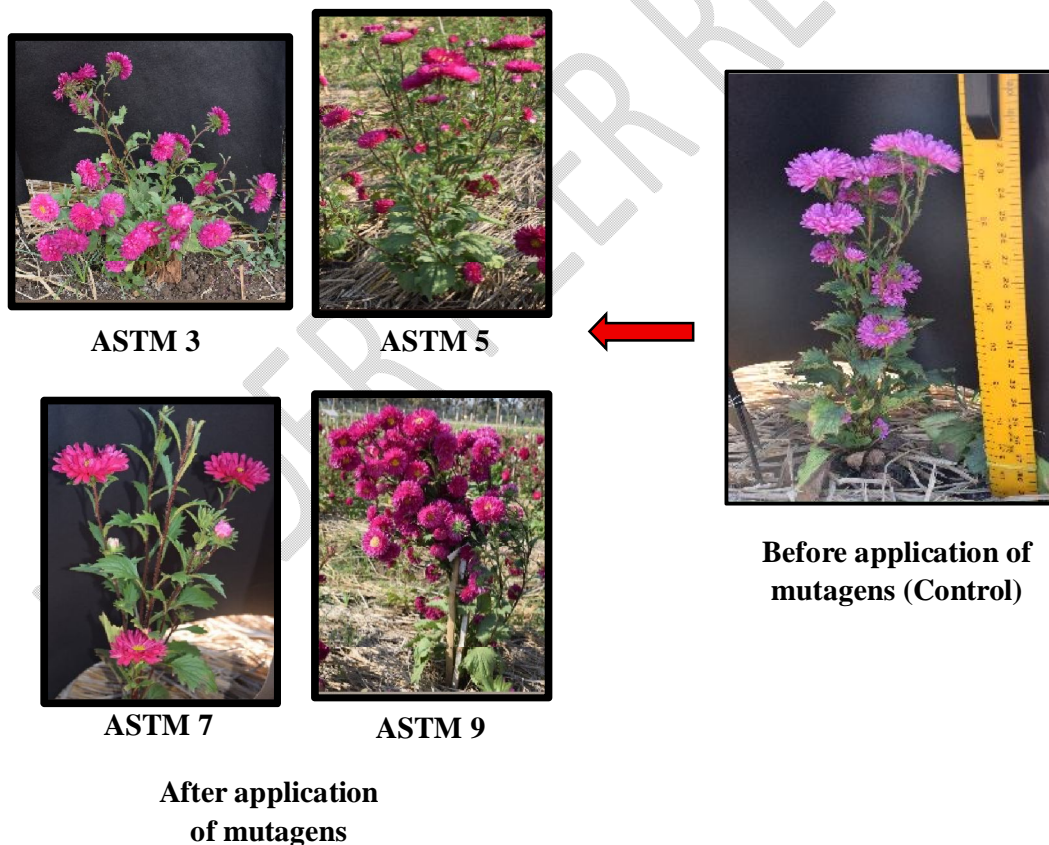


Photo 1: Performance of Phule Ganesh Pink with the application of mutagens

Conclusion

~~On the basis of~~Based on the results obtained from the investigation, it can be concluded that application of physical and chemical mutagens ~~gave significantly influenced the good performance. DES @ 0.15 % gave the maximum in~~ vegetative growth of China aster cv. Phule Ganesh Pink among the treatments, DES at 0.15% proved to be the most effective, resulting in the maximum ease of plant height and number of primary branches. This study highlights the potential of using DES as a mutagen to enhance the growth and development of China aster, providing valuable insights for future horticultural practices and mutation breeding programs.

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