# Genetic Improvement of some Economic Traits of (*Calendula officinalis* L.) Using Sodium Azide and Gamma Rays

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**Abstract:** This study was conducted to evaluate the mutagenic effectiveness of sodium azide and gamma rays on *Calendula officinalis* L. Seeds were treated with four concentrations of sodium azide (250, 500, 1000, and 1500 mg/L) and four doses of gamma irradiation (10, 20, 30, and 40 Gy) beside the untreated seeds (control). Results showed that sodium azide significantly enhanced vegetative traits, including plant height, stem diameter, herb fresh and dry weight, especially at 1000mg/L. Floral traits, such as inflorescence number, size, and weight, increased with sodium azide at 1000 and 1500 mg/L. Gamma radiation, particularly at 10 Gy, increased plant height, stem diameter, herb fresh weight, herb dry weight, and floral characters, including number of inflorescences, number of circumferences in inflorescences, diameter, and fresh weight of inflorescences. Overall, both sodium azide and gamma rays effectively enhanced plant growth, floral characteristics, and productivity, with varying degrees of response across generations. The results of heritability showed moderate to high values for most characters under study in different mutagens in M1 and M2 generations.

***Keywords:*** *Calendula officinalis* L., Mutations, Sodium azide, Gamma rays, Genetic improvement, Heritability.

## INTRODUCTION

*Calendula officinalis* Linn., commonly known as pot marigold, is a well-known ornamental and medicinal plant belonging to the Asteraceae family, with a history of use spanning thousands of years (Sharma and Kumari, 2021). It is a genus comprising 12 to 20 species of annual or perennial herbs (Moghaddasi and Kashani, 2012). Induced mutagenesis has become a widely used technique in biological sciences. It is crucial for expanding the genetic base of germplasm in plant breeding and serves as a valuable tool in functional genomics. Induced mutagenesis has successfully enhanced various ornamental plants, such as *chrysanthemum*, *gerbera*, and *gladiolus*, leading to changes in flower and leaf characteristics (color, shape, or size) as well as physiological traits, such as flowering time and stress tolerance (Bhat *et al.* 2007). Sodium azide (SA) is a well-known heavy metal enzyme that influences on metabolism and respiration of living cells. Sodium azide has become an essential tool in enhancing crop traits, particularly in developing resistance in susceptible plants to harmful pathogens, thus improving yield and quality. Among the various mutagens available for crop improvement, SA plays a significant role in generating point mutations in plant genomes, leading to the production of proteins with altered functions compared to normal plants. The resulting mutant plants exhibit improved survival under stress, increased yield, longer shelf life, and reduced agronomic input compared to non-mutated plants (Khan *et al*., 2009). Kaur and Singh (2024) studied the effects of SA and DES on *Calendula officinalis* L. cv. calypso orange. They noticed that the best vegetative traits, such as plant height, leaf number per stem, stem number per plant, and main stem diameter, were achieved with SA at 1500 ppm. Further research by El-Nashar and Asrar (2016) confirmed that lower concentrations of SA and DES (1000–5000 ppm) improved seed germination, plant height, leaf area, flowering time, inflorescence diameter, and gas exchange parameters on *Calendula officinalis L.* plants. In a study on *Chrysanthemum morifolium* (L.) cv. Maghi, Mostafa *et al.* (2019) treated the seeds with SA at concentrations of 0.12 and 0.18% for 6 and 12 hours. The results showed significant effects on plant height, number of branches, flower head diameter, and flower head weight compared to controls. The SA-treated plants exhibited higher phenotypic and genotypic coefficients of variation (PCV and GCV), suggesting that the treatment increased genetic variability for several traits.

Gamma rays are the most energetic form of such EM radiation, having the energy level from around 10 keV to several hundred kiloelectron volts, and therefore they are more penetrating than other radiation, such as alpha and beta rays (Kovacs and Keresztes, 2002), There are substantial evidence that ionizing radiation, such as gamma ray affects plant growth and vigor by causing morphological, biochemical, and cytological changes in cells and tissues through the generation of free radicals (Aly *et al*., 2022). Gamma rays irradiation has been used for a long time to induce beneficial physiological and genetic changes in plants. This method provides a way to develop desirable traits that may be absent in plants or have been lost over time (Hosseini *et al*., 2023). Shafiei *et al*. (2019) treated leaf explants from three of the most widely cultivated chrysanthemum varieties with different doses of gamma rays. The results indicated that a gamma ray dose of 25 Gy was optimal for inducing mutations in the varieties tested (purple and pink cultivars) and produced the highest number of colored flowers. Results showed that gamma irradiation led to a reduction in plant survival, height, number of flowers heads, stems per plant, stem diameter, and leaves per plant when the rooted cuttings of the chrysanthemum variety ‘Otome Pink’ were exposed to gamma radiation at doses of 0, 10, 15 and 20 Gy (Kumari *et al*., 2013). The objective of the present study was to investigate the effect of

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sodium azide (chemical mutagen) and gamma rays (physical mutagen) on the vegetative, floral characters, and heritability among some traits in induced genotypes of *Calendula officinalis* L.

## MATERIALS AND METHODS

**Plant material and mutant treatments:** This experiment was carried out at the Nursery of Horticulture Department, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt, from 2019 to 2021. The seeds of pot marigold (*Calendula officinalis* L.) were obtained from the El-Kasaseen Research Station, Department of Medicinal and Aromatic Plant Breeding, Horticulture. Research Institute, Agriculture Research Center. For the treatment with sodium azide ($NaN_3$; Mw=65.01), seeds of *Calendula officinalis* L. were soaked in different concentrations (0, 250, 500, 1000, 1500 mg/L) for two hours before planting. Regarding the treatment with gamma rays, the *Calendula officinalis* L. seeds were packed in polyethylene bags and exposed to different doses (0, 10, 20, 30, and 40 Gy) of γ-irradiation from a cobalt 60 ($^{60}Co$) source (Gamma cell 220- Canada). The temperature and dose rate for all the samples were 25±1ºC and 0.33Gy/Sec, respectively. The irradiation treatments were performed at the Egyptian Atomic Energy Authority, National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The treated seeds, along control (untreated seeds), were sown to arise the M1 generation in the nursery during September 2019 to produce the M1 generation. The M2 seeds were raised by selfing M1 plants for each genotype. The seeds collected from the different plants of each treatment were bulked to give rise to the M2 generation. In the second season, the bulked seeds of each M1 treatment and untreated seeds were planted in September 2020 in the tray 84 until the seedlings reached 10 -15 cm in height in November 2020 and then transferred to the field. In both seasons, the experiment was conducted using a simple randomized complete blocks design (RCBD) with three replicates. In each replicate, seedlings were cultured in rows at a cultivation distance of 25-30 cm between plants and 40-50 cm between the lines. At the beginning of flowering, the plants and their flowers were monitored. During both generations, representative plants from each treatment were selected based on their vegetative and floral characteristics. These plants were enclosed with white netting to ensure isolation, and they were self-pollinated to produce the seeds. Subsequently, the seeds were harvested and stored in a dry location for further planting and further studies. In both seasons, all plants were provided with standard agricultural practices as needed from sowing to harvest. Plant height (cm), inflorescence weight (%) values were recorded in M1 and M2generations. stem diameter (mm), herb fresh weight (g), herb dry weight (g), number of inflorescences/plant, diameter of inflorescences (cm), number of inflorescence circumferences/plant, fresh weight of inflorescences/plant (g), diameter of disc flowers (cm) and percentage weight of ray flowers to

**Statistical analysis:** Experimental data were analyzed using analysis of variance (ANOVA) with Co-Stat statistical software, Version 4.2 (Cohort Program, 1990). Pairwise means were compared using the least significant differences (LSD) test at p=0.05. The genotypic and phenotypic variances (Vg and Vp) were estimated using the method of Singh and Choudhary (1985), Broad sense heritability (h2) was calculated using a computer program Agri-stat-Statistical package for analyzing data, as described by Allard (1960).

## RESULTS

**Effect of sodium azide concentrations on the vegetative growth of *Calendula officinalis* L. at M1 and M2 generations:** Analysis of variance for mutagen (sodium azide) showed different significant effects on plant height of *Calendula officinalis* L. in both generations Table (1). In the M1 generation, plant height increased with higher sodium azide concentrations. The 1000 mg/L treatment produced the tallest plants (94.9 cm). This was significantly higher than the control (81.8 cm). In the M2 generation, the pattern continued, where the concentration of 1000 mg/L yielded the tallest plants (130.6 cm), followed by the 1500 mg/L treatment (125.9cm). For the stem diameter, sodium azide also showed a positive response effect on stem diameter, as presented in (Table 1). The 1000 mg/L concentration produced the thickest stems in M1 (20.4 mm) and M2 (22.6 mm). Interestingly, the 1500 mg/L treatment resulted in the thickest stems (29.1 mm) in M2 generation. However, stem diameter at higher concentrations did not always correspond with plant height, suggesting that stem diameter is not always proportional to overall plant growth.

The herb fresh weight data presented in Table (1) showed that sodium azide treatments resulted in significant increases in herb fresh weight in the M1 generation. The 1000 mg/L concentration yielded the highest herb fresh weight (246.9 g), which was significantly greater than the control (139.5 g). On the other hand, in the M2 generation, the 1500 mg/L concentration resulted in an even higher herb fresh weight (1061.6 g), surpassing the 1000 mg/L treatment. However, the increase in fresh weight between these two concentrations was less pronounced than the corresponding difference in dry weight. These results suggest that sodium azide, particularly at 1000 mg/L, plays a substantial role in enhancing fresh herb biomass across both M1 and M2 generations.

The results in Table (1) showed that the sodium azide at 1000 mg/L significantly produced the heaviest dry weight of herb in M1, (107.3 g) compared to the control without sodium azide, which gave (53.0 g). Similar results were noticed in the M2 generation, where the treatment with 1000 mg/L produced the highest dry weight of herbs (271.5 g). However, the control obtained the lowest dry weight of herbs (95.5 g). Interestingly, the 1500 mg/L of sodium azide produced the highest fresh and dry weight of herb, as 1061.6g and 291.4 g, respectively.

**Table (1): Effect of sodium azide concentrations on the vegetative growth of *Calendula officinalis L.* at M1 and M2 generations**

| Generations | Sodium azide (mg/L) | Plant height (cm) | Stem diameter (mm) | Herb fresh weight (g) | Herb dry weight (g) |
|---|---|---|---|---|---|
| First generation (M1) | control | 81.8 | 16.2 | 139.5 | 53.1 |
| | 250 | 84.2 | 15.0 | 150.8 | 70.5 |
| | 500 | 93.6 | 19.3 | 206.0 | 85.2 |
| | 1000 | 94.9 | 20.4 | 246.9 | 107.3 |
| | 1500 | 86.6 | 17.7 | 236.8 | 94.9 |
| L.S.D at 5% | | 4.9 | 3.7 | 46.2 | 13.5 |
| Second generation (M2) | control | 87.9 | 17.2 | 366.4 | 95.5 |
| | 250 | 109.2 | 19.9 | 686.9 | 176.7 |
| | 500 | 122.9 | 20.6 | 735.5 | 207.2 |
| | 1000 | 130.6 | 22.6 | 911.4 | 271.5 |
| | 1500 | 125.9 | 29.1 | 1061.6 | 291.4 |
| L.S.D at 5% | | 6.9 | 4.4 | 175.7 | 67.4 |

**Effect of sodium-azide concentrations on the floral characters of *Calendula officinalis* L. at M1 and M2 generations:** Table (2) shows the effects of sodium azide on the number of inflorescences per plant in the M1 and M2 generations of *Calendula officinalis* L. In the M1 generation, a noticeable increase was recorded in the number of inflorescences as the concentration of sodium azide treatments increased, with 1000 mg/L and 1500 mg/L yielding the highest numbers at 218.8 and 198.8, respectively. The results interestingly demonstrated that the highest treatment (1500 mg/L) decreased the number of inflorescences compared to 1000 mg/L. This finding may be due to the effect of high concentrations, causing metabolic imbalances that reduce the number of flowers. In the M2 generation, the 1500 mg/L treatment yielded the highest number of inflorescences (324.2), representing a significant increase compared to the control (135.3). The number of circumferences in inflorescences/ plant (Table-2) shows considerable variation among sodium azide concentrations. In M1 generation, plants exposed to sodium azide at concentrations of 1000 mg/L and 1500 mg/L exhibited significantly more circumferences (6.5 and 9.6, respectively) compared to the control, which had 3 circumferences. In M2 generation, the number of circumferences further increased, particularly at 1500 mg/L, where the number of circumferences reached 11.7, significantly surpassing the control, which had 3 circumferences. For the diameter of inflorescences, the results in Table (2) showed that sodium azide treatments led to significant increases in inflorescence diameter. In the M1 generation, a 1500 mg/L concentration resulted in the largest diameter (8.6 cm) compared to 6.7 cm in the control. In the M2 generation, the same was observed, where the 1000 mg/L and 1500 mg/L treatments produced diameters of 8.8 cm and 8.7 cm, respectively. These values were notably higher than the control (6.9 cm). Moreover, Table (3) shows the different effects of sodium azide doses on the diameter of disc flowers in M1 and M2 generations.

In the M1 generation, significant increases in the diameter of disc flowers were observed in response to sodium azide treatments. The 1500 mg/L concentration led to the largest disc flowers (2.2 cm), significantly greater than the control, which had a diameter of 1.4 cm. In the M2 generation, these enhancements in disc flower diameter were maintained and, in some cases, further improved. The largest disc flowers were recorded in the 1500 mg/L treatment (2.1 cm), which were significantly larger than the control (1.3 cm).

Fresh weight of inflorescences was affected by different concentrations of sodium azide in both generations, as shown in Table (2). In M1, the fresh weight of inflorescences increased with sodium azide treatment, particularly at the 1000 mg/L and 1500 mg/L concentrations, where the weights were 6.5 g and 7.4 g, respectively, compared to 1.6 g in the control. This significant increase in fresh weight may be attributed to the rise in flower size. In M2, the fresh weight of inflorescences continued to increase during all sodium azide treatments, with the 1500 mg/L treatment producing the heaviest inflorescences (8.4 g), significantly surpassing the control (1.9 g).

Regarding the percentage of the ray flower weight to inflorescence weight, data in Table (2) reveal that the 1000 mg/L and 1500 mg/L treatments showed the highest percentages of the ray flowers (59.2% and 61.5%, respectively) in M1 generation, compared to the control (54.4%). This result suggests that sodium azide at higher concentrations not only enhances the size and number of flowers but also promotes the development of the ray flowers, which are crucial for attracting pollinators. In M2, the ray flower percentage increased, especially at 500 mg/L concentration (64.4%). Interestingly, while the ray flower percentage in M2 was high overall, no significant difference was recorded between the 1000, 1500 mg/L, and the control.

**Table (2): Effect of sodium azide concentrations on the floral characters of *Calendula officinalis L.* at M1 and M2 generations**

| Generations | Sodium azide (mg/L) | Number of inflorescences/ plants | Number of circumferences in inflorescences | Diameter of inflorescences (cm) | Diameter of disc flowers (cm) | Fresh weight of inflorescences (g) | % of the ray weight flowers to inflorescences weight |
|---|---|---|---|---|---|---|---|
| First generation (M1) | control | 124.4 | 3.0 | 6.7 | 1.4 | 1.6 | 54.4 |
| | 250 | 166.0 | 3.3 | 7.6 | 1.6 | 2.5 | 53.9 |
| | 500 | 174.2 | 4.2 | 7.8 | 1.8 | 4.2 | 56.3 |
| | 1000 | 218.8 | 6.5 | 8.1 | 2.1 | 6.5 | 59.2 |
| | 1500 | 198.8 | 9.6 | 8.8 | 2.2 | 7.4 | 61.5 |
| L.S.D at 5% | | 35.7 | 3.2 | 0.5 | 0.2 | 0.8 | 3.9 |
| Second generation (M2) | control | 135.3 | 3.0 | 6.9 | 1.3 | 1.9 | 55.9 |
| | 250 | 232.4 | 6.3 | 8.3 | 1.7 | 4.2 | 56.3 |
| | 500 | 243.1 | 10.0 | 8.4 | 2.0 | 6.7 | 64.4 |
| | 1000 | 294.2 | 10.7 | 8.8 | 2.0 | 8.4 | 60.1 |
| | 1500 | 324.2 | 11.7 | 8.7 | 2.1 | 8.4 | 60.2 |

**Effect of gamma rays doses on the vegetative growth of *Calendula officinalis* L. at M1 and M2 generations:**

As shown in Table 3, the plant height was significantly increased at a lower dose of gamma rays. In the M1 generation, the highest plant height (93.1 cm) was obtained at 30 Gy, which was significantly higher than the control (81.8 cm). In the M2 generation, a different trend was noticed, where the 10 Gy produced the highest plant of 137.1 cm compared to the control (87.7 cm). However, increasing the dose to 40 Gy, slightly decreases the plant height to 104.7 cm in the M2.

The stem diameter followed a similar trend to that of plant height (Table 3). In M1 the highest stem diameter of (24.8 mm) was recorded with the 40 Gy, while in M2, the treatment with 10 Gy, significantly produced the highest stem diameter (25.9 mm) compared to the control (17.2 mm). However, at higher gamma doses (30 and 40 Gy), a decline in the stem diameter was noted.

Data presented in (Table 3) clearly showed that the fresh weight of the herb exhibited a similar pattern of response to gamma radiation in both generations. Moderate doses produced a slight increase followed by a decline at higher concentrations. In M1, the highest herb fresh weight (208.2 g) was recorded at 30 Gy, compared to (139.5 g) for the control. In M2, herb fresh weight reached its peak at 10 Gy (918.8 g), more than twice that of the control (366.4 g). However, as the gamma doses increased, the herb fresh weight decreased. At 40 Gy, the herb fresh weight was reduced to 440.7 g.

It is clear from the data in **(Table 3)** that the herb dry weight in M1 and M2 generations followed a similar pattern to fresh weight. This was because the dry weight of the herb also increased at lower doses of gamma radiation in both generations. In M1, the highest dry weight (98.9 g) was observed at 30 Gy, significantly higher than the control (53.0 g). In M2, the dry weight of the herb was notably higher at 10 Gy (316.5 g) than in the control (95.5 g). However, the dry weight decreased at higher gamma ray doses, with 40 Gy resulting in a dry weight of 197.7 g.

**Table (3): Effect of gamma rays doses on the vegetative growth of *Calendula officinalis L.* at M1 and M2 generations**

| Generations | Gamma rays (Gy) | Plant height (cm) | Stem diameter (mm) | Herb fresh weight (g) | Herb dry weight (g) |
|---|---|---|---|---|---|
| First generation (M1) | Control | 81.8 | 16.2 | 139.5 | 53.0 |
| | 10 | 83.8 | 18.4 | 159.9 | 77.3 |
| | 20 | 83.8 | 20.0 | 198.9 | 93.3 |
| | 30 | 93.1 | 20.9 | 208.2 | 98.9 |
| | 40 | 93.1 | 24.8 | 199.7 | 93.8 |
| L.S.D at 5% | | 5.1 | 2.8 | 51.6 | 16.1 |
| Second generation (M2) | control | 87.7 | 17.2 | 366.4 | 95.5 |
| | 10 | 137.1 | 25.9 | 918.8 | 316.5 |
| | 20 | 132.8 | 23.6 | 668.5 | 230.8 |
| | 30 | 115.7 | 21.1 | 606.9 | 213.7 |
| | 40 | 104.7 | 20.7 | 440.7 | 197.7 |

Table (4) shows a significant increase in the number of inflorescences with increasing doses of gamma rays. In the M1 generation, the control plants produced 124.43 inflorescences, while the 40 Gy doses produced the highest number (211.33). In the M2 generation, the number of inflorescences increased at lower doses of gamma radiation. The 10 Gy dose resulted in the highest number of inflorescences (437.13), more than three times higher than the control (135.33). This result suggests that gamma radiation not only has immediate effects in the M1 generation but also induces lasting effects that influence flower production in next generations. However, at higher doses of 30 and 40 Gy, the number of inflorescences declined (266.80 and 207.90, respectively), indicating that excessive radiation may have negative effects on flower production in the second generation. In the M1 generation, the number of circumferences in inflorescences increased with higher gamma rays doses (Table 4). The control produced an average of 3 levels, while the 40 Gy doses resulted in the highest number (10.30 circumferences). The doses of 10, 20, and 30 Gy resulted in an intermediate increase in the number of circumferences (6.77, 7.33, and 8.23, respectively). In the M2 generation, the number of circumferences in inflorescences significantly increased at the 10 Gy dose (12 levels), demonstrating a strong positive effect of gamma radiation at low doses on floral complexity. However, at higher doses of 20, 30, and 40 Gy, the number of circumferences in inflorescences particularly decreased at the highest dose (5 circumferences).Concerning the inflorescence diameter, the control produced relatively smaller ones (6.73 cm), while the 40 Gy doses resulted in the largest inflorescences (8.60 cm). Treatments with (10, 20, and 30 Gy) led to an increase in inflorescence diameter (7.97 cm, 8.33 cm, and 8.43 cm, respectively). In the M2 generation, the control produced an inflorescence diameter of 6.90 cm. However, the 10 Gy concentration produced the largest inflorescences (9.07 cm). As the radiation dose further increased (20, 30, and 40 Gy), the diameter of inflorescences decreased (8.43, 7.77 and 7.13 cm, respectively).

Data presented in Table (4) show an increasing trend with higher gamma rays doses with the diameter of disc flowers in the M1 generation. The control produced disc flowers with an average of 1.40 cm. in diameter. In the M2 generation, the diameter of disc flowers increased at the lower doses of gamma radiation; the 20 Gy doses produced the largest diameter (2 cm). This suggests that the effects of gamma radiation on disc flower size are more pronounced in the second generation. However, higher doses (10, 30, and 40 Gy), the diameter of disc flowers decreased slightly (1.97 cm, 1.40 cm, and 1.23 cm, respectively).

The fresh weight of inflorescences showed a positive correlation with the dose of gamma rays in the M1 generation, as shown in Table 4. The control had the least fresh weight, at 1.60 g, while the 40 Gy doses produced the heaviest inflorescences (5.33 g). The 10, 20, and 30 Gy doses also resulted in significant increases in fresh weight. In the M2 generation, the fresh weight of inflorescences increased at the 10 Gy doses, reaching 8.83 g, more than four times the weight of the control (1.87 g). This result indicates that moderate radiation doses significantly enhance the biomass of flowers in the second generation. However, higher radiation doses (20, 30, and 40 Gy) led to a decrease in fresh weight, with the 40 Gy dose producing the lowest fresh weight (2.60 g). Table 4 shows that the percentage weight of the ray flowers to inflorescence weight significantly increased with higher gamma ray' doses in the M1 generation, where the increase reached 62.77% with the 40 Gy In the M2 generation, the 10 Gy dose resulted in the highest ray flower percentage (64.3%). at doses (20, 30, and 40 Gy), the percentage of the ray flowers did not increase significantly.

**Table (4): Effect of gamma rays doses on the floral characters of *Calendula officinalis* L. at M1 and M2 generations**

| Generations | Gamma rays (Gy) | Number of inflorescences | Number of circumferences in inflorescences | Diameter of inflorescences (cm) | Diameter of disc flowers (cm) | Fresh weight of inflorescences (g) | % weight of the ray flowers to inflorescences weight |
|---|---|---|---|---|---|---|---|
| First generations (M1) | Control | 124.4 | 3.0 | 6.7 | 1.4 | 1.6 | 54.4 |
| | 10 | 181.2 | 6.8 | 7.9 | 2.0 | 4.3 | 57.1 |
| | 20 | 199.4 | 7.3 | 8.3 | 2.1 | 4.6 | 59.5 |
| | 30 | 199.5 | 8.2 | 8.4 | 2.2 | 5.0 | 61.3 |
| | 40 | 211.3 | 10.3 | 8.6 | 2.2 | 5.3 | 62.8 |
| L.S.D at 5% | | 25.1 | 2.6 | 0.4 | 0.2 | 1.5 | 3.6 |
| Second generations (M2) | Control | 135.3 | 3.0 | 6.9 | 1.3 | 1.9 | 55.9 |
| | 10 | 437.1 | 12.0 | 9.1 | 1.9 | 8.8 | 64.3 |
| | 20 | 325.9 | 7.3 | 8.4 | 2.0 | 5.7 | 56.7 |
| | 30 | 266.8 | 5.5 | 7.8 | 1.4 | 3.4 | 56.4 |
| | 40 | 207.9 | 5.0 | 7.1 | 1.2 | 2.6 | 57.4 |
| | | 76.6 | 1.9 | 0.5 | 0.4 | 1.6 | 8.2 |

**Phenotypic (P.C.V) and genotypic (G.C.V) coefficient of variability for the vegetative characters of *Calendula officinalis L.* at M1 and M2 generations:** Data in Table (5) reveal that the values of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were low, and the heritability ($h^2$) was moderate for this trait in M1 generation for all treatments. However, all these values in the M2 generation were moderate. The genetic control over plant height was found, although environmental influence could still be present. However, in the M2 generation, gamma rays outperformed sodium azide significantly in improving plant height. Gamma rays treated plants exhibited impressively high heritability at 92.8%, indicating that most of the observed variation was genetically controlled, with minimal environmental influence. This finding was further supported by the high genetic advance (GA) of 39.7, suggesting that selection for plant height in the gamma rays treated population would be highly effective. The variability in stem diameter, PCV of 15.5, and a GCV of 10.7, suggested a noticeable environmental influence and low heritability. Sodium azide treatment in the M2 generation led to increased genetic stability, with PCV and GCV rising to 22.2 and 19.5, respectively. Heritability improved significantly to 76.8%, and genetic advance (GA) reached 7.7, indicating enhanced genetic gain. In contrast, gamma ray treatment demonstrated a more consistent and stronger genetic effect on both generations. In the first generation, high PCV (17.1) and GCV (15.5) values were recorded, along with a heritability of 81.6%, showing that most of the phenotypic variation was genetically controlled. These values remained high in the M2 generation (PCV: 16.5, GCV: 14.6), with heritability at 78.9% and GA at 5.8, slightly lower than sodium azide but still substantial. The statistics showed that the fresh weight of herbs has a significant level of variability. The high estimates of phenotypic and genotypic coefficient of variation (> 20%) were recorded for herb fresh weight in the M1 generation with sodium azide treatments, indicating that a large proportion of the phenotypic variation was genetic in origin. The heritability ($h^2$) was relatively high at 78.5% suggesting that selection for this trait would be moderately effective even in M1. However, in the M2 generation, the PCV and GCV rose to 36.3 and 34.1 respectively, showing a strong increase in genetic variability. Importantly, heritability climbed to 88.3% and the genetic advance (GA) reached 496.21, the highest among all traits studied. In comparison, gamma ray treatment had a different pattern. In the M1 generation, the PCV was 20.6 and the GCV was 14.0, reflecting a more modest level of induced variability. Heritability in M1 was also quite low. However, similar to sodium azide, the M2 generation showed marked improvement. PCV and GCV values increased to 37.7 and 35.1, respectively. Heritability improved substantially to 86.7% and the genetic advance was 403.7, indicating that gamma rays did succeed in inducing beneficial mutations, though to a lesser extent than sodium azide. Sodium azide and gamma rays treatments significantly affected the herb dry weight, with improvements more pronounced in the M2 generation. In M1, sodium azide resulted in closely aligned PCV (26.7) and GCV (25.2) values, indicating minimal environmental influence and high heritability (89.3%). In M2, variability increased (PCV 40.2, GCV 36.3), and although heritability slightly decreased to 81.7%, it remained high. The rise in genetic advance (GA 140.9) confirmed enhanced and heritable genetic gains. Gamma ray treatment followed a different trend. In M1, PCV and GCV were slightly lower (24.0 and 21.7), with heritability at 81.7%. However, in M2, gamma ray induced greater genetic variability (PCV 38.8, GCV 36.9) with minimal environmental influence, as reflected in the closely aligned values. Although the value of the phenotypic coefficient of variation (PCV) for herb dry weight was found to be higher than the genotypic coefficient of variation (GCV), the differences were small in magnitude. This result suggests a lower influence of environment on the expression of this character, and reflects high estimates of heritability, 90.8%, and GA 152.7.

**Phenotypic (P.C.V) and genotypic (G.C.V) coefficient of variability for the floral characters of *Calendula officinalis* L. at M1 and M2 generations:** The statistics showed that most of the analyzed features (PCV), (GCV), heritability (h2), and genetic advance (GA) have a significant level of variability in the number of inflorescences across M1 and M2 generations. Sodium azide treatment in M1 showed a PCV of 22.1 and GCV of 19.3, with moderately high heritability (76.3%), indicating that most of the variation was genetic. In M2, its effect intensified, with PCV and GCV increasing to 30.6 and 28.8, respectively, heritability rising to 88.8%, and genetic advance (GA) reaching 137.4, confirming stable and heritable improvements. The gamma rays also showed strong genetic effects. In M1, PCV and GCV were 19.8 and 18.4, with even higher heritability (86.5%) than sodium azide, suggesting stronger genetic control early on. In M2, gamma rays had the most pronounced effect, with PCV and GCV surging to 43.6 and 41.0, respectively, heritability remaining high at 88.47%, and the highest GA recorded at 218.2, but gamma rays exhibit superior potential, particularly in the M2 generation, making it a highly valuable tool for boosting flower productivity in *Calendula officinalis* L.

Results also revealed that heritability estimates ranged in M1 generation from 68.8% for sodium azide to 77.3% for gamma rays. Moreover, in M2 generation, it has varied from 87.9% for sodium azide to 92.2% for gamma rays. Most heritability in both generations ranged from moderate to high, indicating that a relatively large portion of the phenotypic variance was due to genetic causes. Replace with "Comparing treatments revealed the expected genetic advance with sodium azide (6.7), and the genetic advance remained high with gamma rays at 6.7, nearly identical to sodium azide. Finally, from the above-mentioned, it could be concluded that there is a difference in the response of the *Calendula officinalis* L. plants to each

**Table (5): Phenotypic (P.C.V) and genotypic (G.C.V) coefficient of variability for the vegetative characters of *Calendula officinalis L.* at M1 and M2 generations**

| Traits | Treatments | First generation (M1) | | | | | Second generation (M2) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Vp | Vg | P.C. V | G.C. V | $h^2$% | Vp | Vg | P.C.V | G.C.V | $h^2$% | GA |
| Plant height | Sodium azide (mg/L) | 37.6 | 30.9 | 6.9 | 6.3 | 82.1 | 307.1 | 293.7 | 15.2 | 14.9 | 95.6 | 34.5 |
| | Gamma rays (Gy) | 32.0 | 24.8 | 6.4 | 5.7 | 77.4 | 432.3 | 400.9 | 17.9 | 17.3 | 92.8 | 39.7 |
| Stem diameter | Sodium azide (mg/L) | 7.6 | 3.6 | 15.5 | 10.7 | 47.9 | 23.6 | 18.2 | 22.2 | 19.5 | 76.8 | 7.7 |
| | Gamma rays (Gy) | 11.8 | 9.6 | 17.1 | 15.5 | 81.6 | 12.8 | 10.1 | 16.5 | 14.6 | 78.9 | 5.8 |
| Herb fresh weight | Sodium azide (mg/L) | 2800.6 | 2197.9 | 27.0 | 23.9 | 78.5 | 74413.9 | 65709.2 | 36.3 | 34.1 | 88.3 | 496. |
| | Gamma rays (Gy) | 1395.3 | 644.9 | 20.6 | 14.0 | 46.2 | 51099.7 | 44301.9 | 37.7 | 35.1 | 86.7 | 403.7 |
| Herb dry weight | Sodium azide (mg/L) | 481.6 | 429.9 | 26.7 | 25.2 | 89.3 | 7009.7 | 5728.8 | 40.2 | 36.3 | 81.7 | 140.9 |
| | Gamma rays (Gy) | 400.7 | 327.3 | 24.0 | 21.7 | 81.7 | 6673.3 | 6056.5 | 38.8 | 36. | 90.8 | 152.7 |

*** Vp= phenotypic variances; Vg=Genotypic variances; P.C.V= phenotypic coefficient of variation; G.C.V= genotypic coefficient of variation; h2 = heritability and GA= genetic advance.**

mutagen, which may be due to differential mutagen-sensitivity. Further confirming that the induced variability is highly heritable and can be exploited in selection. Results also revealed that heritability estimates ranged in M1 generation from 68.8% for sodium azide to 77.3% for gamma rays. Moreover, in M2 generation, it has varied from 87.9% for sodium azide to 92.2% for gamma rays. Most heritability in both generations ranged from moderate to high, indicating that a relatively large portion of the phenotypic variance was due to genetic causes. Replace with "Comparing treatments revealed the expected genetic advance with sodium azide (6.7), and the genetic advance remained high with gamma rays at 6.7, nearly identical to sodium azide. Finally, from the above-mentioned, it could be concluded that there is a difference in the response of the *Calendula officinalis* L. plants to each mutagen, which may be due to differential mutagen-sensitivity. Further confirming that the induced variability is highly heritable and can be exploited in selection.

Data presented in Table (6) show that the diameter of inflorescences for *Calendula officinalis* L., was affected by gamma rays and sodium azide in both generations. Considering the phenotypic coefficient of variation, values ranged in M1 generation from 9.1 for sodium azide to 9.6 for gamma rays. Moreover, in M2 generation, PCV varied from 9.7 for sodium azide treatments to 11.62 for gamma rays. GCV ranged from 8.4 for sodium azide to 9.3 for gamma rays in M1 generation. Moreover, in M2 generation, GCV varied from 9.2 for sodium azide to 11.1 for gamma rays. A high value of heritability (84.9%) for sodium azide, and a high value (92.7%) was recorded for gamma rays in the M1 generation. In M2 generation, heritability varied from 88.6% for sodium azide to 91.0% for gamma rays. Among the expected genetic advances, the highest value was recorded by gamma rays (1.7), and the lowest value (1.5) was obtained from sodium azide. Both sodium azide and gamma rays significantly influenced the genetic parameters of disc flower diameter in *Calendula officinalis* L. in M1 and M2 generations. Sodium azide showed strong genetic control with a PCV of 18.9 and GCV of 18.3 in the M1 generation, indicating minimal environmental influence, supported by very high heritability (92.9%). In M2, PCV remained relatively stable at 19.46, GCV slightly declined to 16.7, resulting in a moderate drop in heritability to 73.6% and a genetic advance (GA) of 0.5, suggesting that although selection remained effective, its efficiency was somewhat reduced. Gamma rays had a comparable effect in M1, with PCV and GCV at 17.9 and 16.7, respectively, and high heritability (87.1%), indicating substantial genetic influence. However, in the M2 generation, gamma rays induced the greatest variability, with PCV and GCV rising to 25.4 and 22.5, respectively, the highest values recorded for this trait, along with heritability at 78.5% and the highest GA at 0.7. These results indicated that both mutagens were effective in enhancing disc flower diameter, but gamma rays were more potent in the M2 generation, producing greater genetic variability and stronger potential for selection.Table (6) present that sodium azide and gamma rays had varying effects on the percentage weight of ray flowers to inflorescence weight in *Calendula officinalis* L. In the M1 generation, sodium azide resulted in PCV and GCV values of 6.4 and 5.3, respectively, with a heritability of 67.53%, indicating moderate to high genetic control and suggesting that the trait is largely heritable with limited environmental influence. These values remained consistent in the M2 generation, with PCV at 6.6, GCV at 5.4, heritability slightly declining to 65.6 %, and genetic advance (GA) at 5.3, confirming stable and moderately heritable variation suitable for selection. Gamma rays showed similar genetic control in M1, with PCV and GCV at 6.2 and 5.3, and slightly higher heritability at 73.1%, indicating strong potential for early selection. In the M2 generation, although PCV increased to 8.5, GCV dropped significantly to 4.2, resulting in a sharp decline in heritability to 23.8% and a reduced GA of 2.0, highlighting increased environmental influence and diminished selection potential. Overall, sodium azide maintained consistent moderate genetic control across both generations, while gamma rays were more effective in M1 but less reliable in M2.

## DISCUSSION

The present results indicate that using different doses of two mutagenic agents, sodium azide and gamma rays, affects the economic characteristics of pot marigold (*Calendula officinalis* L). There were clear differences in the responses of vegetative and floral traits among seeds treated with sodium azide and gamma rays. These results have been confirmed by several studies, including Badr *et al*. (2000) on *Tagetes erecta*, El-Elfeky *et al*. (2014), Phanindra *et al*. (2018) on *Helianthus annuus* L., Mostafa *et al*. (2019) on *Chrysanthemum morifolium, El*-Khateeb *et al.* (2022) on *Helichrysum bracteatum* L., El-Nashar and Asrar (2016), and Kaur and Singh (2024) on *Calendula officinalis* L. who reported that the treatment with 1000 and 1500 mg /L of sodium azide increased the vegetative and flowering traits such as plant height, stem diameter, herb fresh weight, number of inflorescences/plants, number of rings in inflorescences, diameter of inflorescences and fresh weight of inflorescences on *Calendula officinalis* L compared to untreated plants. On the other hand, the results showed that all gamma rays doses significantly increased vegetative and floral characters in M1 and M2 generations. The treatment of 30 Gy had the highest of herb fresh and dry weight in M1 generation, but the highest stem diameter, inflorescences characters including number of inflorescences, number of circumferences per inflorescence, inflorescence diameter, diameter of disc flowers, fresh weight of inflorescences and percentage weight of the ray flowers relative to inflorescence weight, were observed at 40 Gy treatment. The results indicated that gamma radiation not only increased the total number of inflorescences but also promoted the development of more complex inflorescence structures, potentially

**Table (6): Phenotypic (P.C.V) and genotypic (G.C.V) coefficient of variability for the floral characters of *Calendula officinalis* L. at M1 and M2 generations**

| Traits | Treatments | First generation (M1) | | | | | Second generation (M2) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Vp | Vg | P.C.V | G.C.V | $h^2$% | Vp | Vg | P.C.V | G.C.V | $h^2$% | GA |
| Number of inflorescences | Sodium azide (mg/L) | 1516.8 | 1157.5 | 22.1 | 19.3 | 76.3 | 5642.9 | 5009.6 | 30.6 | 28.8 | 88.8 | 137.4 |
| | Gamma rays (Gy) | 1313.1 | 1135.7 | 19.8 | 18.4 | 86.5 | 14338.8 | 12686.1 | 43.6 | 41.0 | 88.5 | 218.2 |
| Number of circumferences in inflorescences / plant | Sodium azide (mg/L) | 9.5 | 6.5 | 57.7 | 47.9 | 68.8 | 13.5 | 11.9 | 44.4 | 41.7 | 87.9 | 6.7 |
| | Gamma rays (Gy) | 8.4 | 6.5 | 40.7 | 35.7 | 77.3 | 12.3 | 11.3 | 53.5 | 51.3 | 92.2 | 6.7 |
| Diameter of inflorescences | Sodium azide (mg/L) | 0.5 | 0.4 | 9.1 | 8.4 | 84.9 | 0.6 | 0.6 | 9.7 | 9.2 | 88.6 | 1.5 |
| | Gamma rays (Gy) | 0.6 | 0.6 | 9.6 | 9.3 | 92.7 | 0.8 | 0.8 | 11.6 | 11.1 | 91.0 | 1.7 |
| Diameter of disc flowers | Sodium azide (mg/L) | 0.1 | 0.1 | 18.9 | 18.3 | 92.9 | 0.2 | 0.1 | 19.5 | 16.7 | 73.6 | 0.5 |
| | Gamma rays (Gy) | 0.1 | 0.1 | 17.9 | 16.7 | 87.1 | 0.2 | 0.1 | 25.4 | 22.5 | 78.5 | 0.7 |
| Fresh weight of inflorescences | Sodium azide (mg/L) | 6.4 | 6.2 | 57.3 | 56.3 | 96.7 | 9.4 | 7.4 | 51.9 | 46.2 | 79.3 | 5.0 |
| | Gamma rays (Gy) | 2.6 | 2.0 | 38.8 | 34.1 | 77.2 | 8.5 | 7.8 | 64.7 | 61.9 | 91.7 | 5.5 |
| % weight of ray flowers to inflorescence weight | Sodium azide (mg/L) | 13.4 | 9.1 | 6.4 | 5.3 | 67.5 | 15.4 | 10.1 | 6.6 | 5.4 | 65.6 | 5.3 |
| | Gamma rays (Gy) | 13.5 | 9.9 | 6.2 | 5.3 | 73.1 | 24.6 | 5.9 | 8.5 | 4.2 | 23.8 | 2.4 |

*Vp= phenotypic variances; Vg=Genotypic variances; P.C.V= phenotypic coefficient of variation; G.C.V= genotypic coefficient of variation; h2 = heritability and GA= genetic advance.

enhancing reproductive success and contributing to the overall expansion of the floral structure. In the same trend, Kaur *et al*. (2017) and Abou Dahab *et al*. (2023) on *Calendula officinalis* L. plants, found that the highest dose of gamma rays (40 Gy) was the most effective for increasing the vegetative and floral traits Conversely, in the M2 generation, the tallest plants, along with the highest stem diameter, herb fresh weight, dry weight and inflorescence characteristics, were increased at lower gamma doses compared to the control. These findings align with those reported by Stepanenko and Regir (1982) on *Calendula officinalis* L. and Singh *et al*. (2009) and Sarhan *et al*. (2019), who observed that vegetative and floral characters in *Tagetes erecta* L. in M2 generation decreased with increasing radiation dose. The range of phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h2), and genetic advance (GA) was tested to determine the extent to which genetic factors cause the observed variations. In the M1 generation, sodium azide had a strong and immediate impact, with exceptionally high PCV (57.26) and GCV (56.31), indicating minimal environmental influence and very strong genetic control, supported by the highest heritability observed (96.73%). Sodium azide particularly effective for early-generation selection. Although PCV and GCV slightly decreased in M2 (51.93 and 46.24, respectively), they remained high, and heritability, while reduced to 79.26%, still indicated a strong genetic influence, with a genetic advance (GA) of 5. In contrast, gamma rays showed a moderate effect in M1, with PCV at 38.81, GCV at 34.10, and heritability at 77.20%, suggesting reasonable genetic control but less impact than sodium azide. However, in M2, gamma rays produced the strongest response, with PCV and GCV increasing dramatically to 64.72 and 61.97, respectively, the highest values recorded for this trait- along with very high heritability (91.68%) and the highest GA (5.49), surpassing sodium azide. All treatment of sodium azide and gamma rays showed moderate to high values for most studied characters among different mutagens in the M1 and M2 generations, including various genetic parameters such as plant height, stem diameter, herb fresh weight, herb dry weight, number of inflorescences, number of inflorescences circumferences, inflorescences diameter, inflorescences fresh weight, and the percentage weight of ray flowers relative to inflorescence weight.

## CONCLUSION

Both sodium azide and gamma rays were produced beneficial mutations in Calendula plants by inducing significant changes in vegetative and floral traits, including an increase in inflorescence size, shape, and weight. Such improvements may raise the production value and represent important economic aspects for *Calendula officinalis* L.

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# التحسين الوراثي لبعض الصفات الإقتصادية للأقحوان بإستخدام أزايد الصوديوم وأشعة جاما

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**المستخلص :** أُجريت هذه الدراسة في كلية الزراعة بجامعة قناة السويس (2019-2021)، بهدف دراسة تأثير أزايد الصوديوم ($NaN_3$) وأشعة جاما على الصفات الاقتصادية لنبات الأقحوان. حيث تم تعريض البذور لأربعة تركيزات مختلفة من أزايد الصوديوم (0،250، 500، 1000، 1500 ملجم/لتر) وأشعة جاما (0،10، 20، 30، 40 جراي). تم تصميم تجربة بسيطة في قطاعات عشوائية كاملة (RCBD) بثلاث مكررات عبر الجيلين (الجيل الأول 2020/2019 والجيل الثاني 2021/2020). أظهرت النتائج أن أزايد الصوديوم أدي إلي زيادة النمو الخضري، بما في ذلك ارتفاع النبات وقطر الساق ووزن النبات الطازج والجاف، وخاصةً عند تركيز 1000 ملجم/لتر. وبالنسبة للصفات الزهرية، أدي أزايد الصوديوم إلي زيادة عدد النورات وحجمها ووزنها، حيث أظهرت تركيزات 1000 و1500 ملجم/لتر أفضل النتائج. وكان لأشعة جاما تأثير مماثل للصوديوم أزايد ، وخاصةً عند تركيز 10 جراي، حيث زاد ارتفاع النبات وقطر الساق ووزن النبات الطازج والجاف، وبعض الصفات الزهرية بما في ذلك عدد النورات، عدد المحيطات الزهرية داخل النورات و قطر النورات ووزنها الطازج. وبشكل عام، أدي استخدام كل من أزايد الصوديوم وأشعة جاما نمو النبات والخصائص الزهرية والإنتاجية بشكل فعال، مع درجات متفاوتة من الاستجابة عبر الأجيال. وأظهرت نتائج معدل التوريث قيماً متوسطة إلى مرتفعة لمعظم الصفات التي تم دراستها تحت تأثير المعاملات المختلفة عبر الجيلين الأول والثاني.

***الكلمات المفتاحية :*** *الأقحوان ، الطفرات ، أزايد الصوديوم ، أشعة جاما ، التحسين الوراثي ، معدل التوريث .*