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Induced physical and chemical mutagenesis on Marigold (*Tagetes erecta* L.) to determine the lethality, germination and seedling survivability

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Abstract
The broad range of plant mutants has resulted in the creation of a new variety of plants that includes food, medicinal and ornamental flowering crops. Marigold are commercially and traditionally harvested for economic, cultural and medicinal uses. In this present mutational study, healthy *Tagetes erecta* L. seeds were exposed to gamma rays and EMS with subsequent concentrations. My research findings indicate the seed germination, seedling survival and determination of LD_{50} of the treated seeds alongside the control seeds. The present study reflects that the concentration of mutagen doses is inversely proportional to seed germination and seedling survival. From the above, the lethal dose was calculated and demonstrated that Gamma rays are more efficient mutagen than EMS for *Tagetes erecta* L.

Keywords: mutagen, gamma rays, ems, marigold, plant breeding

Introduction
Marigold (*Tagetes erecta* L.), an indigenous flowering crop of Mexico yet renowned as African Marigold, endowed with a wide range of commercial potentialities in the industrial and medicinal sector. Although, the production of these flowers is being highly demanded throughout the year and commonly used in wreaths, hall decoration, garland making, etc. (Singh *et al.*, 2009) [1]. Being a potent plant with high commercial importance, it exhibits an astonishing way in the field of pharmacology. The phytochemicals extracted from the leaves and flowers are used commercially in making pharmaceutical drugs, colourants in food and cosmetics. The main pigment and one of the major constituents of *Tagetes erecta* L. is lutein. It is an oxy-carotenoid or xanthophyll, containing 2 cyclic end groups (one β and one α- ionone ring) and the basic C-40 isoprenoid structure common to all carotenoids (Priyanka *et al*., 2013) [2].
Mutation breeding, one asset in conventional breeding to develop new varieties apart from hybrids. The changes occur solely in their genetic makeup instead of combining two different genes. However, the practical application for spontaneous mutation rate is too low. Therefore, inducing mutation by physical and chemical mutagens was a marvellous option that opened the door to modern science to overcome the limitation drawn by nature to increase the frequency of mutation and variation in desirable plants in a short period. The Plant breeding era with fundamental science was implemented after the discovery of the Law of Heredity and further advancements were developed to combine a different set of genes from different varieties, i.e., Hybridization. The initiation of genetic alternations in plants was done by Lewis John Stadler in the late 1920s through X-rays on maize and barley and laid a foundation towards mutation assisted plant breeding programme. The genotypic and phenotypic changes are induced using agents, i.e., Mutagens. Besides several methods, the quickest way to tap into the genetic material for altering few characters instead of changing the whole genotype is achieved through induced mutagenesis. Around the globe, 3346 mutant varieties from 228 different plant species were officially released through mutation breeding (FAO/IAEA Mutant Varieties Database, 2021) [3].
Gamma rays (source ^{60}Co) and EMS (alkylating agent) were the mutagens used in this study. The practical application accounts for Gamma irradiations were done commonly in plant mutation breeding programme to increase the frequency of mutation because of its high penetrating capability than other forms of radiations (Kovacs and Keresztes, 2002) [4] by chromosomal reconstitution and deletion while EMS (Ethyl methanesulfonate) considered as the common chemical mutagen among plant breeders which results in mispairing primarily alkylated bases - G/C to A/T transitions. (Sega, 1984; Vogel and Natarajan, 1995) [5, 6] and produces non-lethal point mutations (Bhat *et al*., 2007) [7]. The intensity of both mutagens coupled with efficient targeting of the gene causes high density, wide genomic mutations.
The heterozygosity within the genotype of Marigold with cross-pollination vulnerability and promising pharmacological activities may be a suitable experimental material for physical and chemical mutagenesis.

Materials and Method
The experiment was conducted in the Department of Botany, Annamalai University, Chidambaram, Tamil Nadu. The experiment material comprised of healthy and good quality seeds of Marigold yellow, which were taken for the subsequent mutagenic treatments: Gamma rays (Source ^{60}Co) and EMS (Ethyl methanesulfonate).

Physical Mutagen Treatment
In physical mutagenesis, seeds of Marigold were irradiated with the following concentration 10 KR, 20 KR, 30 KR, 40 KR and 50 KR of gamma rays (source ^{60}Co) at Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam. The treated seeds alongside the control seeds were placed in

a petri-dishes for germination and seedling survivability studies.

Chemical Mutagen Treatment

In chemical mutagenesis, 10 mM, 20 mM, 30 mM, 40 mM and 50 mM solution of ethyl methanesulfonate with 100 ml distilled water was prepared separately. The seeds were presoaked in distilled water for 4 hours at room temperature and after removing the surplus water, presoaked seeds were submerged in the prepared solution of EMS with the mentioned concentration for 8 hours. After chemical treatment, the seeds were rinsed thoroughly 10 times to wash out the surplus chemical residue. The treated seeds alongside the control seeds were placed in a Petri-dishes for germination and seedling survivability studies.

Germination and Seedling Survivability Studies

The germination study was determined at the 15th day to calculate the germination percentage and the lethal dose (LD_{50}) of the control and treated plants. Then, the seedling survival study was determined on 30th day after sowing to calculate seedling survivability percentage.

Result and Discussion

The lethality of the mutagen dose is often determined as the reduction of fifty percent in the population. In the control plant, 94 % of the germination rate showed the highest germination percentage among the treated plants. The uniform concentration with lower to higher doses of gamma rays and EMS show a gradual decrement in the germination of Marigold seeds while tallying their seedling survivability, the seedlings were descended to the minimal after germination, which was calculated on the 30th days after sowing.

Apart from the control plant, the highest germination rate was observed in the lowest concentration whereas the lowest germination rate was observed in the highest concentration, which was significantly reported by previous workers in little millet (Ramkumar and Dhanavel, 2020) [8], *Andrographis paniculata* (Kasthuri and Dhanavel, 2020) [9] and Roselle (Priyanka and Dhanavel, 2020) [10].

The highest germination percentage in gamma irradiated seeds was 86 % (10 KR) whereas in EMS treated seeds, the highest percentage was 82 % (10 mM) and the lowest percentage in gamma was 12 % (50 KR) whereas in EMS, it was 8 % (50 mM). Reduction in germination rate was due to the damage imposed by the mutagenic induction, which simplifies the reasonable answer. The treatment might be responsible for the permutation in cellular constituents at molecular level (Khan and Goyal, 2009) [11] and disturbance in formation of enzyme during germination (Deepika, 2016) [12].

This study evaluated the LD_{50} value by observing 50% reduction in the seed germination, which was noted at 30 KR in gamma irradiated seeds (Table 1) and 30 mM in EMS treated seeds of Marigold (Table 2).

The treatment with 50 KR of gamma rays showed less survival 43.86 % whereas 50 mM of EMS also showed low survival 26.17 %. Similar observations with little variance were reported in gamma irradiated marigold cv double orange seeds (Latha and Dharmatti, 2018) [13], the reasonable answer can be assumed due to the role of environmental factor.

Table 1: Determination of LD_{50} for Gamma Irradiation Treatment

Treatment Doses of Gamma Rays	Seed Germination Percentage (%)	Seedling Survival Percentage (%)	Percent Over Control (%)	Percent of Reduction Over Control (%)
Control	94	98.47	100.00	00.00
10 KR	86	88.68	91.48	8.52
20 KR	62	73.33	65.95	34.05
30 KR	54	64.07	57.44	42.56
40 KR	28	50.16	29.78	70.22
50 KR	12	43.86	12.76	87.24

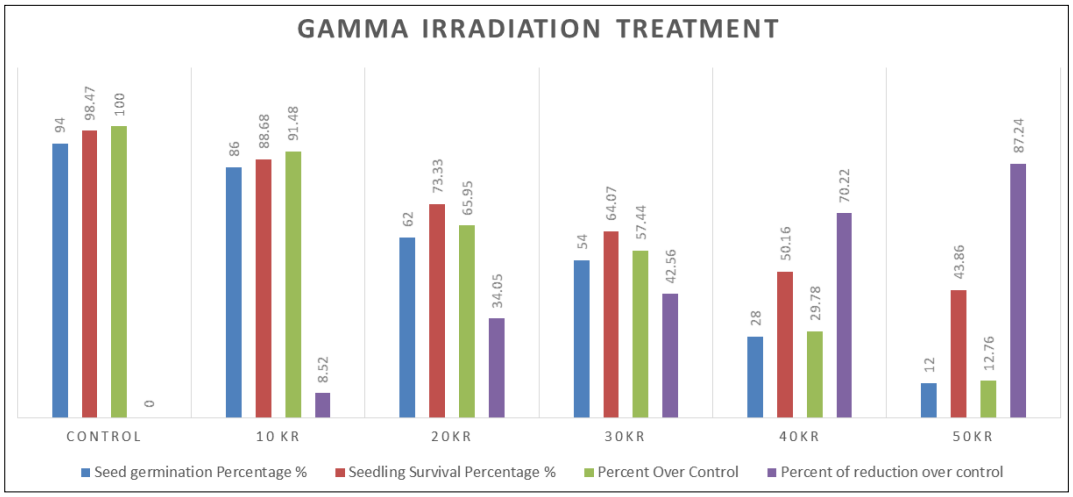

Fig 1: Effect of Gamma Rays in Seed Germination and Seedling Survival Percentage

Table 2: Determination of LD_{50} for EMS Treatment

Treatment Doses of EMS	Seed Germination Percentage (%)	Seedling Survival Percentage (%)	Percent Over Control (%)	Percent of Reduction Over Control (%)
Control	94	98.47	100.00	00.00
10 mM	82	94.86	87.23	12.77
20 mM	66	86.20	70.21	29.79

30 mM	52	78.07	55.31	44.69
40 mM	24	54.54	25.53	74.47
50 mM	8	26.17	8.51	91.49

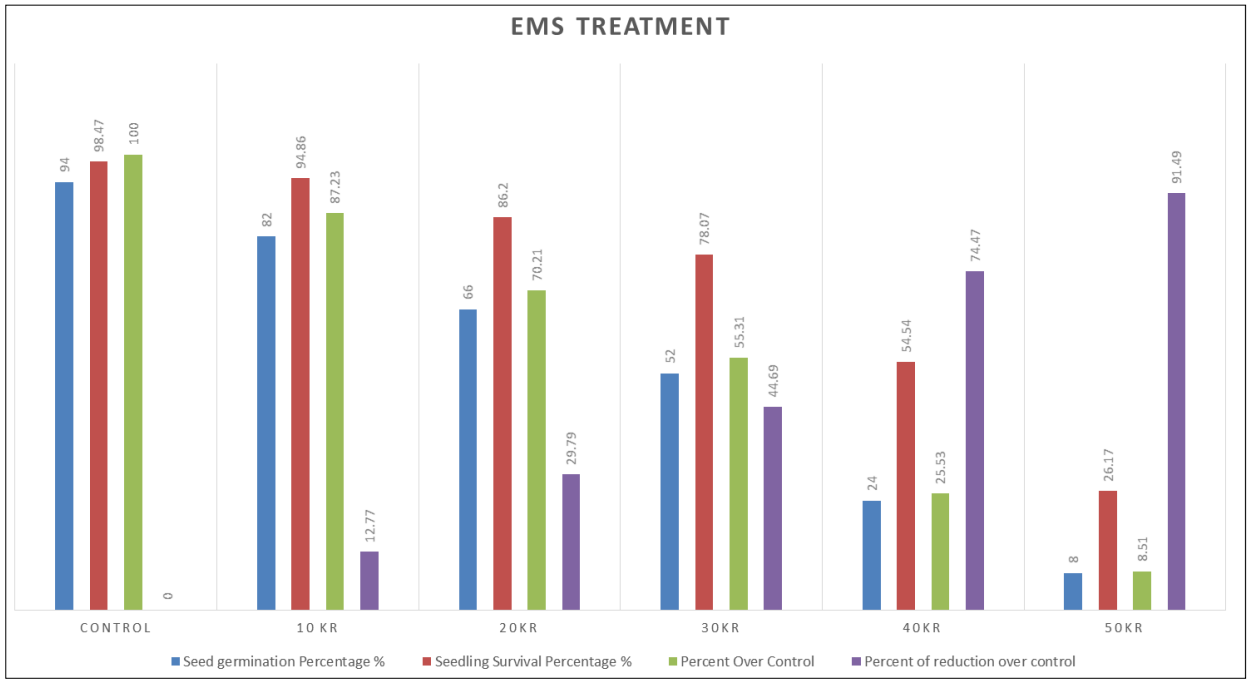

Fig 2: Effect of EMS in Seed Germination and Seedling Survival Percentage

Conclusion

In contrast to the physical and chemical mutagens used, the reduction in seed germination and survivability of EMS was lesser than gamma rays but the lethal dose was observed at 30 KR of gamma rays and 30 mM of EMS. Consecutively, seed germination and seedling survivability decreased with higher doses of mutagens. In accordance to above result, it can be concluded that gamma irradiated seeds can be effectively used for flowering plants in plant breeding programme for better results.

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