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Saniya
Ph.D. (Research scholar), Department of Floriculture and landscape Architecture, UHS, Bagalkot, Karnataka, India

P Pavan Kumar
Assistant Professor (FLA), Technical Officer to Vice Chancellor, UHS, Bagalkot, Karnataka, India

Sarvamangala Cholin
Assistant Professor (GPB), Dept. of CIB, COH, Bagalkot, Karnataka, India

Bapuraygouda Patil
Assistant Professor, Department of Seed Technology, Seed Unit, UHS, Bagalkot, Karnataka, India

Mahesh YS
Assistant Professor, Department of Plant Pathology, AICRP on Potato, Horticulture Research and Extension Center, Somanahallikaval, Hassan

AM Shirol
Associate Professor, Department of FLA, COH, Bagalkot, Karnataka, India

Corresponding Author:
Saniya
Ph.D. (Research Scholar), Department of Floriculture and landscape Architecture, UHS, Bagalkot, Karnataka, India

LD50 value of diethyl sulphate (DES) induced mutagenesis in Nerium

Saniya, P Pavan Kumar, Sarvamangala Cholin, Bapuraygouda Patil, Mahesh YS and AM Shirol

Abstract
Induced mutagenesis using both chemical and physical mutagens is one of the noteworthy breeding tool to enhance variability in existing germplasm. Chemical mutagens such as DES, EMS, MMS and sodium azide have been commonly used to cause a mainstream of practical variations in several ornamental crops. For every mutation breeding experiment, determination of mutagenic sensitivity is an important step as it will vary with every species and varieties. In this investigation, semi-hard-wood cuttings of *Nerium oleander* pink and white genotypes were treated with ten different concentrations of DES ranging from 0.0% (untreated control) to 0.4% DES. The results shown steady and significant reduction in survival of cuttings with increase in dosage of DES. The probit curve analysis based on the survival percentage revealed the LD50 dosage of DES to be 0.3% for both nerium pink and white genotypes.

Keywords: Induced mutation, *Nerium oleander* L. (Pink and white) genotypes, Probit analysis, EMS

Introduction
Nerium oleander is an evergreen shrub belongs to dogbane family Apocynaceae. It is the only species currently classified in the genus *Nerium*. It is commonly known as oleander, from its superficial resemblance to the unrelated olive Olea. It is widely cultivated and thought to be originated from Southwest Asia. This plant is known by many names throughout the world which includes Adelfa, Baladre, *Cascabela thevetia*, Common Oleander, Exile Tree, Laurier-Rose, Nérion, *Nerium indicum*, *Nerium Oleander*, *Nerium odorum*, *Oleander blatter*, Oleandre, *Oleandri folium*, Rose Bay, Rose Laurel and Sweet Scented Oleander. Oleander grows well in warm subtropical regions, where it is extensively used as an ornamental plant in landscapes, in parks, and along roadsides [1]. Oleander is commercially propagated vegetative means by semi-hard-wood cuttings and being a vegetative propagated plant it gives wide scope for application of crop improvement accepts. Crop improvement and development of new varieties plays a vital role in crop production. In floriculture industry there is constant demand for novelty in existing crops. Development of new cultivars through conventional methods like selection and hybridization in ornamental crop have resulted in cultivars of various sizes, flower colors, and shapes but its application nerium has been time consuming due to per and post zygotic barriers in *Nerium oleander*. But until now no attempts have been made though induced mutation to create novel cultivar.

Therefore, mutation breeding is a viable option to enhance genetic variability and to improve economic traits such as floral traits, flowering pattern, plant architecture, *etc*. Induced mutagenesis is a potential plant breeding tool which can induce the generation of allelic variants of genes that modulate the expression of traits. Mutation breeding which leads to altered phenotypes after permanent heritable change in the structure of the genetic material [2], is now established as a time-saving and inexpensive approach for flower crop improvement [3]. Chemical mutagens (DES, EMS, MMS and sodium azide) and irradiation (Gamma rays, X-rays and ion beams) have been widely used to induce a large number of functional variations in ornamental crops. Chemicals induce mainly point mutations while ionizing radiations normally induce chromosomal rearrangements and deletions [4]. Diethyl Sulfate (DES) $C_4H_{10}O_4S$ or $(C_2H_5)_2SO_4$ is a colorless, corrosive, oily liquid that darkens with age and has a faint peppermint odor. It is a possible mutagen and is reasonably anticipated to be a human carcinogen. DES treatments in plant observed various chromosomal associations at metaphase I (trivalents, quadrivalents and also univalents located aside of the plate or near the poles of the microsporocyte).

The univalent formation induced by mutagens supposed to be a result of changes in the structure of chromosomes followed by the reduction of chiasma frequency due to restriction of pairing to homologs [4]. The appearance of the multivalent associations is considered to be caused by mutagen-induced chromosome breaks followed by the reciprocal translocation joining [5]. This chemicals mutagens can cause phenotypic effect as well as genotypic effect in the genomic strands these associations can be a result of chromosome mismatches and breakages which lead to translocations, inversions, point mutations, insertions and or deletions leading to the phenotypic and genotypic changes which could be beneficial for crop plants. Chemical mutagens are in high demand as they increase mutation frequency and are easier to handle, hence ideal and suitable for this research. In order to avoid excessive loss of actual experimental materials, sensitivity tests must be conducted to determine LD50 (*i.e.*, the safe dose at which half population of the planting material survive) doses before massive exposure of similar materials. LD50 dose is considered as the dose at which highest frequency of mutation occurs. With this background, the present investigation was undertaken aiming to determine the optimum lethal dose (LD50) for DES in Nerium (*Nerium oleander* L.).

Materials and Methods

Preparation of planting material

From the mother plants, 10-15 cm length semi hard wood cuttings were taken and leaves were trimmed and slant cut was given just below the nodal portion in two nerium genotypes *i.e.,* (G1-Pink) and (G2-White) respectively.

Mutagenic treatment

Ninety cuttings per treatment were treated with different concentration diethyl sulphate (DES), cuttings were soaked in DES solution of different concentrations *viz.,* 0.0% (Untreated control), 0.1%, 0.2%, 0.3% and 0.4%. After incubation at room temperature for twelve hours, the cuttings were thoroughly rinsed with running tap water for 10 minutes to wash out the chemical residues. Ninety treated cuttings per treatment per replication were planted in nursery polythene bags filled with red soil: FYM: sand (1:1:1) along with untreated cuttings as control. Planted cuttings were placed inside polyhouse (Wherein the temperature was 28–30 °C and relative humidity was 80-85%) for 45 days until rooting of cuttings. The percentage survival was recorded at eight weeks after planting. The experiment was laid out in (RCBD) Randomized complete block design with three replicates. The LD50 value was calculated based on probit analysis using the lethal of treated cuttings to that of control.

Probit analysis

The LD50 values were determined based on Probit analysis [6]. The probit function is the inverse cumulative distribution function (CDF) associated with the standard normal distribution. The LD50 for each genotype was estimated through the simple linear regression model by fitting the straight line equation y= a + bx; where y is the response variable (percent survival), x is the independent variable (irradiation dose), while a and b represent the slope and constant, respectively.

Results and Discussion

Determination of Lethal dose

In present investigation, a steady reduction in survival rate of cuttings with increase in dose of EMS was observed (Table 1). The significance difference was observed between two genotypes and level of mutagen on survival percentage. The survival percentage of nerium genotype (pink) DES treated population ranged from 77.78 per cent (0.1% DES) to 30.45 per cent (0.4% DES). When the dose of DES treatment exceeds 0.4%, none of the treated cuttings survived. In case of nerium genotype (white) DES treated population, survival percentage ranged from 73.88 per cent (0.1% DES) to 32 per cent (0.4% DES). When the dose of DES treatment exceeds 0.3%, none of the treated cuttings survived.

Table 1: Effect of DES on survival of cuttings in nerium pink and white genotypes

Treatment	Treatment details	Nerium genotypes		
		Survival percentage (%)	Per cent survival over control (%)	Per cent reduction over control (%)
T1.	Untreated control (Pink)	90	100	
T2.	DES 0.1% + Genotype-1 (Pink)	77.78	86.42	13.57
T3.	DES 0.2% + Genotype-1(Pink)	71.66	79.62	20.6
T4.	DES 0.3% +Genotype-1 (Pink)	65.55	72.83	27.16
T5.	DES 0.4% + Genotype-1 (Pink)	30.45	33.83	66.16
T6.	Untreated control (White)	90	100	
T7.	DES 0.1% + Genotype-2 (White)	73.88	82.08	17.91
T8.	DES 0.2% + Genotype-2 (White)	62.21	69.12	30.87
T9.	DES 0.3% + Genotype-2 (White)	59	65.55	34.44
T10.	DES 0.4% + Genotype-2 (White)	32	35.55	64.44
	S.E(m)	3.5		
	CD(5%)	12.54		

*G1-(Nerium pink),G2-(Nerium white)
EMS-(Ethyl Methane Sulfonate)

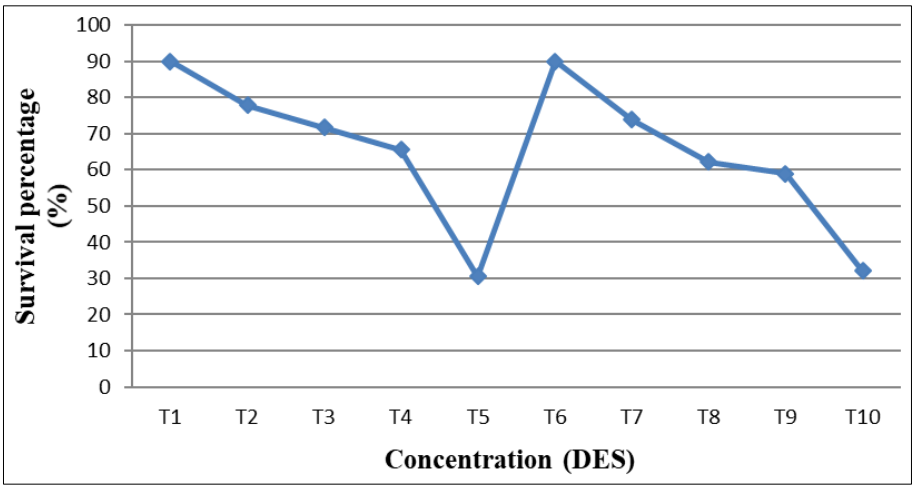
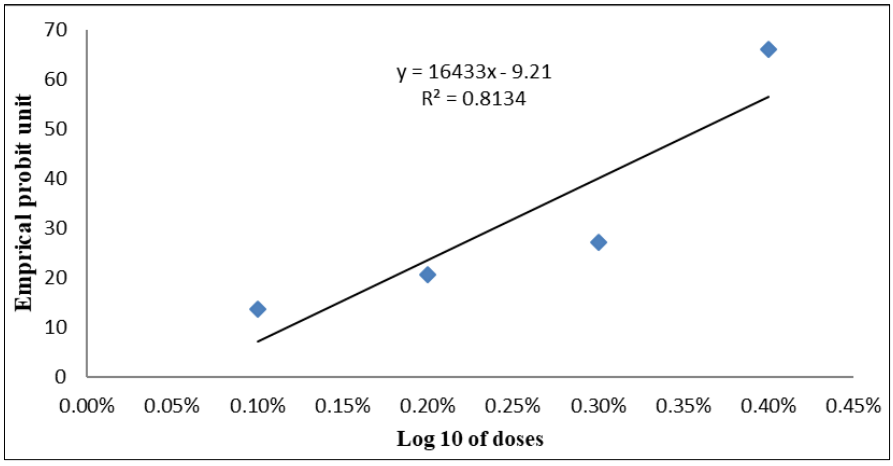
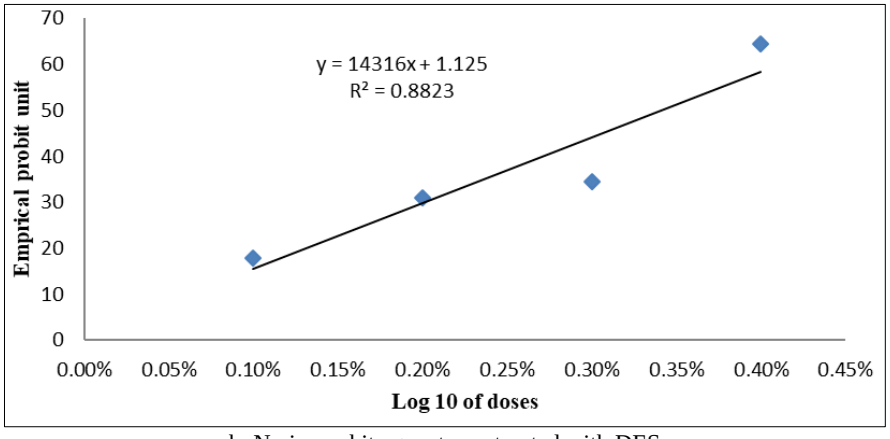


Fig 1: Graphical representation of survival percentage (%) of nerium pink and white genotypes with respect to DES



a. Nerium pink genotypes treated with DES



b. Nerium white genotypes treated with DES

Fig 2: Probit curve for the determination of LD50 value for DES in nerium pink and white genotypes

LD50 for DES was fixed based on survival percentage in two nerium genotypes. Probit analysis was carried out based on survival rate of two nerium genotypes (pink and white) cuttings after treatment with different doses of DES compared with that in untreated control (Fig 1). In the present study, LD50 value for DES as assessed from the probit curve analysis and the results are illustrated in (Fig 2a-b). From the results it was found that the LD50 value for the two nerium genotypes *viz.*, of pink and white was (0.3% DES) for both genotypes.

Different ex plant and level of mutagen administrated have created variations in flower plant species. High dose application of mutagen will increase mutation frequency in induced mutation studies to create genetic diversity. Among the chemical mutagens DES has especially been demonstrated to be the most potent mutagen. Similar results of decline in the survival percentage with increase in DES concentration have been reported in *Gloriosa superba* cv. Andhra wild [7] and in *Calendula officinalis* L [8]. To identify the biological influences of different chemical mutagens, LD50 value is

mostly utilized as an guide. It has been shown that a linear dependency exists between survival percentage and the dosage of chemical mutagens. Observations of the current study are in agreement with the above reports in chrysanthemum variety 'Maghi' [9] using DES on the seed germination and seedling survival.

Conclusion

The present investigation showed that based on survival percentage, the LD50 values for DES was found to be 0.3% for both nerium pink and nerium white genotypes respectively. These optimum mutagen doses determined for the nerium genotypes could be useful for mutation breeding studies in nerium crop improvement. This kind of study is first time reported in nerium cultivars as per literature available.

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References

1. Farooqui S, Tyagi T. *Nerium oleander*: It's application in basic and applied science: A review. Int. J Pharm. Pharm. Sci. 2018;10(3):1-4.
2. Rego LV, Faria RT. Tissue culture in ornamental plant breeding review. Crop Breeding. Appl. Biot. 2001;1:285-300.
3. Datta SK, JAT, Da Silva. Role of induced mutagenesis for development of new flower colour and type in ornamentals. Floriculture, ornamentals and plant biotechnology: Advances and topical issues. Global Science Books Ltd., Middlesex, U.K. 2006;1:640-645.
4. Zeerak NA. Cytogenetical effects of gamma rays and ethyl methane sulphonate in tomato (*Lycopersicon esculentum* var. *cerasiforme*). Phytomorphology. 1992;42:81-86.
5. Nashar YI. Effect of chemical mutagens (sodium azide and diethyl sulphate) on growth, flowering and induced variability in *Amaranthus caudatus* L. and *A. hypochondriacus* L. Dissertation, University of Egypt, 2006.
6. Finney DJ. Statistical Method in Biological Assay. Charles Griffin Co; c1978.
7. Padmapriya S, Rajamani K. Median lethal dose estimation of physical and chemical mutagens in *Gloriosa superba* L. J. P. P. 2019;8(5):627-631.
8. Samatadze TE, Zoshchuk SA, Hazieva FM, Yurkevich OY, Svistunova NY, Morozov AI, *et al*, Phenotypic and molecular cytogenetic variability in calendula (*Calendula officinalis* L.) cultivars and mutant lines obtained *via* chemical mutagenesis. Scientific Report, 2019.
9. Kapadiya DB, Chawla SL, Patel AI, Ahlawat TR. Exploitation of variability through mutagenesis in chrysanthemum (*Chrysanthemum morifolium* Ramat.) Var. Maghi. The Bioscan. 2014;9(4):1799-1804.