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Comparative study on the effect of chemical mutagens of sodium azideand di-ethyl sulfate on improving morphological traits and yield components of Borgo officinalis L, plant

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***Abstract*---**Borage seeds were soaked in different doses of sodium azide (SA) and di-ethyl sulphate (DES), at 0.1,0.2,0.3 and 0.4% for 6 hrs., M2 seeds were harvested from M1 plants.In MG1 it was found that most SA doses increased plant height and stem thickness. SA at 0.3% & 0.4%, increased the ramification of main branches and enhanced leaves formation, leaf area, FW and DW of leaves, DW /FW ratios and root length. SA 0.3% was more effective than 0.4% and SA 0.2% increased the root number. Whereas, in MG2, SA 0.3 % significantly increased most morphological traits, but stem thickness responded well to SA at 0.1% and No. of branches was increased with SA 0.4 %. A great improvement in the formation of leaves obtained with SA 0.3%.The leaves DW/FW ratios, showed a positive response to SA 0.1 and 0.3% Concerning DES effects in MG1 and 2, the results in MG1revealed that all doses increased plant height and stem diameter and 0.4% DES produced the tallest and highest No. of branches and at 0.1% gave thickest stems . Applying DES 0.3% greatly improved the formation of leaves and the largest leaf area was recorded with 2000 ppm DES. Data in MG2, indicated that DES 0.3% improved the ramification of main branches. The doses of 0.2-0.4% increased No. of leaves/ plant, producing the largest leaves at 0.2%, whereas, the low dose resulted the heaviest weights of leaves, whereas , the high dose improved roots formation. In MG1and MG2, all doses of SA and DES increased the seed yield,(except SA 0.1% in MG1).The highest seed

set % was recorded at DES 0.1% and the lowest one with SA 0.1%. SA at 0.2 % was the most effective in increasing seed yield. In MG1 Chl.a increased with SA at 0.3%and DES 0.2%, Chl. b recorded the lowest value by SA 0.1%, carotenoids increased with SA at 0.4% and DES at 0.2 and 0.3%, all SA reduced the carbohydrates % in leaves. In MG2, the carbohydrates % decreased with all doses, the lowest one was in SA at 0.2%. The fixed oil content of seeds in MG1 recorded the highest value with DES at 0.2% and 0.4% in MG2. DNA banding patterns using RAPD-PCR under effect five mutants.RAPD assay: using five mutagen namley; SA 0.2 %; SA 0.4%; DES 0.1, 0.3%and 0.4%. Using ten RAPD- PCR technique revealed ninety one variables bands, were of them 61 polymorphic bands, with unique bands by 67% of polymorphism and 30 of them monomorphic bands under effect five mutants SA 0.2 %; SA 0.4%; DES 0.1,0.3 and 0.4%.The comparison between SA vs DES, indicated that soaking borage seeds in DES at 0.3% showed higher mutagen efficiency on most vegetative growth traits of than SA and control.

***Keywords*---**chemical mutagens, SA, DES, RAPD-PCR, Borage.

Introduction

Medicinal and aromatic plants are important sources of plant secondary metabolites for human health care. Borage (*Borago officinalis* L.,family Boraginaceae) is an annual medicinal plant native to Europe, North Africa, and Asia Minor, (Beaubaire and Simon, 1987). It is cultivated as crop vegetable, pharmaceutical herb and industrial properties. It is outbreeding, although evidence of borage self-compatibility (Montaner *et al.*, 2000), it is one of the best known sources of gamma linolenic acid (GLA),so it has a great potential market interest due to its content of gamma linolenic acid(GLA) which is extracted from seeds. The leaves and flowers contain mucilage, saponins, essential oil, alkaloid, vit . C, Ca and K (Sayanova *et al.*,1999, Belch and Hill, 2000 and Gupta and Singh, 2010). The plant is used as nerve and cardiac tonic and a home remedy for blood purification (Kybal, 1980), as is considered useful to treat asthma, bronchitis, cramps, diarrhea, antispasmodic,anti-hypertensive and kidney ailments (Usmanghani *et al.*, 1997 and Duke *et al.*, 2002). Owing to its content of unsaturated fatty acids, remarkable antioxidant property , the oil of borage has been reported to lower serum cholesterol,phospholipids and triglyceride rates (Gu *et al.*,1998), and increases the rates of polyunsaturated fatty acids in the plasma, liver, aorta and renalartery tissues (Harbige *et al.*,2000 and Rio-Celestino *et al.*, 2008), it is rich in GLA (26%-38%), fatty acids (linoleic acid (35%-38%), oleic acid (16%-20%), palmitic acid (10%-11%), stearic acid (3.5%-4.5 %), after evening primrose plant, these plants together are recognized as the important national sources of GLA, but borage is the preferred source The oil content of seeds is 30–40% by weight , of which 20-30% is GLA, about twice the level in evening primrose oil (Beaubaire and Simon, 1987). Moreover, borage leaves and leaf petiole is used in pickles and salads, they have a high nutritive value (Badi *et al.*, 2008).

Borage plant has a multifactorial self-incompatibility system, so self-pollination is ineffective for breeding and pollinating insects are required, besides the variability in borage crop is very narrow. Conceivably, mutation breeding (MB) may help to produce a new, uniform varieties and creating variability through chemical mutagens to improve this crops. Recently, MB has become increasingly popular as an effective tool for crop improvement (Acharya *et al.*, 2007), it is an important and potential approach for enhancing the genetic variation and direct improvement for desired traits and certain qualitative and quantitative characters, to produce higher yields and active components. Agrawal and Kumar (2021) stated that several chemical mutagens have proven their importance in plant mutation breeding. During the last several years, MB has been successfully utilized for the improvement of many medicinal and aromatic plants, Kulkarni and Baskaran (2003), on periwinkle obtained a high alkaloid producing variety. Slavova *et al.*, (2004) obtained a high yielding varieties with high essential oil content of peppermint, oiganum, verbascum and lavandula through MB. There are several mutagens are available and used for crop improvement ,each one has its important role (positive or negative)on the crops, but the most important mutagens (sodium azide (SA), Tri-methanolamine (TMA), di-ethylamine (DEA) and di-ethyl sulphate (DES), EMS, MMS, nitrogen mustard which are being used to improve yield and quality traits ,most of these chemicals were applied at the range from :0.005 to 4.0%. (Fahad and Khan, 2001).We used two chemical mutagens(namely; sodium azide (SA) and De-ethyle sulfate(DES), in this study. Sodium azide (SA- NaN_3) is an ionic compound, the mutagenicity of SA is mediated by an organic metabolite (L-azidoalanine) and generated by O-acetylserine sulfhydrylase enzyme which enters the cell nucleus and interacts with the DNA, creating point mutations (Gruszka *et al.*, 2012). Diethyl sulfate (DES) it is an alkylating agent ($(C_2H_5)_2SO_4$), that react with the DNA structure through the phosphate group and nitrogen bases ,which used to Induce mutation by alterations in DNA molecules as transitions, transvers ions, deletions, insertions, inversions, DNA single and double strand breaks, and DNA recombination (Acquaah, 2007). It used as chemical mutagenesis to improve the agronomic traits of crops, its effect depends on the permeability of seed coat and nature of the mutagens, it provide a good scope for selection to enhance the variability of characters. Important findings were obtained on the effects of both SA and DES on improvement of many important medicinal and aromatic plants (Elfeky *et al.*, 2014) on *Helianthus annuus* and (Bashir *et al.*, 2013) on fenugreek indicated that mutagenic effectiveness of SA decreased with the increase in dose. Iqbal *et al.*, (2019) studied SA effect on *Nigella sativa*.

Regarding effects of DES, EL-Keltawi *et al.*, (1985) on marjoram used DES at (0.005- 4.0%) for 4 h , obtained six mutants in MG_1 and MG_2 differed significantly compared with the control. Bhat *et al.*, (2007) observed that DES and SA the induction of meiotic aberrations was higher in DES than SA, suggesting that DES could be more effective in inducing additional variability than SA. Gulfishan *et al.*, (2011) on *Capsicum annuum* obtained various meiotic abnormalities and reductio in chiasma frequency in the DES-treated plants, the highest % recorded at 0.05% DES. Also, Gulfishan *et al.*, (2012) found that DES at 0.05% decreased the pollen fertility from 94.80% (control) to 63.35 %. On chilli plants, Sanjai Gandhi *et al.*, (2014) found that increasing DES level decreased the values of morphological and yield characters (plant height, No. of branches, days to first

flowering, No of fruits /plant, No of seeds/fruit and seed weight. There was an increase in the level of steroidal sapogenin in *Trigonella corniculata* with DES treatment (Kolakar *et al.*, 2018). On glory lily (*Gloriosa superba*), Padmapriya and Rajamani (2019) recorded decrease in germination %, plant height, No. of leaves with the increase in dosage of DES. The objective of this study was comparative study on the effect of chemical mutagens of sodium azide and di-ethyl sulfate on improving morphological traits and yield components of *Borgo officinalis* L, plant.

Materials and Methods

A field experiment was conducted at the Experimental Nursery of the Ornamental Hort., Dept., Fac. of Agric.,Cairo Univ., Egypt, during two successive mutation seasons of 2019/2020 and 2020/2021 ($MG_1$and MG_2), aiming at studying the response of borage (*Borago officinalis* L. plants) to chemical mutagens (sodium azide(SA) and di-ethyl sulphate(DES),to evaluate their effects on flowering, seed yield, oil yield ,photosynthetic pigments and genetic variation (RAPD analyses).

Plant Materials

Borago officinalis L. local cv. Purple seeds were kindly obtained from the Dept. of Med.& Aromatic Plants, Min.of Agric.,Egypt. Filter-sterilized solutions (10%) of NaN_3 and DES were prepared in deionized water, then they were diluted with sterile 0.1 M phosphate buffer to give the four concentrations (0.1%, 0.2%, 0.3% and 0.4%) of each mutagens.

Treatments of SA and DES

The seeds were presoaked in distilled water (wet seeds) for six hours, and then they were subjected to the prepared four concentrations of SA and DES solutions for six hours. One set of seeds was kept without any treatment (untreated, as control). After 6 hrs. of soaking, the seeds were thoroughly washed in tap water .

Planting seeds

On 3rd Nov., 2019 (MG1, the first mutative generation), the treated seeds were sown in the open field in clay loam soil (Table, A), each treatment contained three rows, at 50 cm apart and 35 cm between the seed hills within the row(24000 plants/fed). The plants were fertilized every two months with a dose of 2 g/plant of N-P-K (Life Green: 20-20-20).The plants were grown under field conditions and irrigated every 7-10 days as the plants needed. The second mutative generation (MG2)

The mass selection of plants (MG1-generation) was run from April to June, 2020, where plants of each treatment were evaluated and selected, in order to obtain the (MG_2)seeds, according to Sinhamahapatra and Rakshit (1990). Observations were made throughout the periods of vegetative and flowering. At maturity (May- June), the seeds of all treatments (MG1) were harvested separately. Selection under field conditions was done in SA and DES treated plants (bulk seeds of the top high

seed yielding plants) to raise MG2.,as the seeds (MG2) were sown on 20th Oct., 2020 in open field, as in the first generation.

Experimental design

The layout of the experiment was a complete randomized block design, with 9 treatments, 3 replicates (each replicate contained 5 plants). Agricultural practices: All the recommended cultural practices were carried out during the plant growth and flowering period, irrigation was done out regularly as the soil needed.

Data recorded

The following data on vegetative growth, flowering characters and as chemical composition, were recorded for in M1 and M2 for treated plants with sodium azide and di-ethyl sulphate: Plant height ,main stem diameter , No.of branches and leaves/plant, fresh and dry weights of leaves/plant ,leaf area ,,No.of roots and root length,No.of inflorescences and seed yield/plant., pigments ,, carbohydrates and oil content in seeds(%). Determination of chemical constituents: Photosynthetic pigments: he contents of chlorophyll a, b and total carotenoids were determined in leaf samples (mg/g fresh matter) according to (Saric *et al.*, 1967).The content of total carbohydrates (%) in dried leaves, were determined according to (Herbert *et al.*, 1971).

Determination of oil content in seeds

Solvent extraction: The oil extraction from ground seeds of Borago Officinalis L. was performed according to the Soxhlet extraction method, where n-hexane was used as extraction solvent. The ratio of sample to solvent was 1:10. (Bhatnagar and Krishna, 2013). Oil content (%); the percentage of oil content in borage seeds was calculated according to the method described by A.O.C.S, Firestone (2009), using the following equation: Where: W is the weight of obtained oil in (g) and Ws is the weight of seeds in (g)., and recorded then the percentage of fruit set was calculated as the following equation according to Westwood (1978) as follows: Fruit set (%) = Number of set fruitlets × 100 Number of flowers at full bloom.

Table A. The physical and chemical characteristics of the soil

pH	EC $ds.m^{-1}$	Soluble Cations and Anions (meq/L)							
		Cations				Anions			
		Na	K	Ca	Mg	Cl	HCO_3	CO_3	SO_4
7.2	2.76	0.59	0.49	1.03	0.65	1.2	0.44	0.04	1.08
Particle size distribution									
Total Sand				Silt		Clay		Texture	
34.8				34.0		31.2		Clay loam	

Statistical analysis

Data recorded on vegetative growth, flowering, seeds yield and chemical composition traits were statistically analyzed. Analysis of variance (ANOVA) was carried out, and the means were compared using L.S.D. test at the 5% level, as described by (Snedecor and Cochran, 1980).

Molecular Studies

Aimed to determine genetic markers and the phylogenetic tree between different varients of ten DNA. Procedure for isolation of genomic DNA. Grind 100 mg fresh plant tissue (or 20 mg dry plant tissue) to fine powder in liquid nitrogen. Transfer it to a 1.5 ml tube. Add 600 µl Buffer PCB and 12 µl of β-mercapto ethanol to the sample, and mix thoroughly . Incubate at 65°C for 25 min. Add 20 µlRNaseA (20 mg/ml), mix and incubate for 2 min at room temp. Then ,add 0.6 ml of chloroform to the tube, mix well by inverting 10 times. Centrifuge at 12,000 x g for 2 min. Carefully transfer the supernatant (400 µl) to a clean 1.5 ml tube.Add 200 µl Buffer BD, mix thoroughly, then add 200 µl ethanol (96-100%), mix thoroughly Transfer the mixture (including any precipitate) into the EZ-10 column placed in a 2 ml collection tube. Centrifuge at 9,000 x g (12,000 rpm) for 1 min. Discard the flow-through. Add 500 µl PW Solution, and centrifuge for 1 min at 9,000 x g (12,000 rpm). Discard the flow-through, then Add 500 µl wash solution, and centrifuge for 1 min at 9,000 x g (12,000 rpm). Place the empty column in the micro centrifuge and centrifuge for an additional 2 min at 9,000 x g (12,000 rpm) to dry the EZ-10 membrane. Discard flow-through and transfer the spin column to a clean 1.5 ml centrifuge tube. This centrifugation step ensures that no residual ethanol will be carried over during the following elution. Add 50-100 µl TE Buffer directly onto the center part of EZ-10 membrane.

PCR- Amplification of RAPD (Amplification reaction)

It was carried out in 25µl reaction mixture contained 2µl of genomic DNA, 3µl of the primer, 2.5µl of 10X Taq DNA polymerase reaction buffer, 1.5 units of Taq DNA polymerase and 200 mm of each dNTPs. The following PCR program was used in a DNA. Initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 42°C for 90 sec. for annealing tem, 72°C for 90 Sec. and final extension at 72°C for 2 min. Products by RAPD- PCR were separated on 1.5% agarose gels in 1X TAE buffer and detected by staining with ethidium bromide according to Sambrook *et al.*, (1989). DNA ladder 100bp was used and PCR products were visualized by UV-trans-illuminator and photographed by gel documentation system, the amplified bands were scored as (1) for presence and (0) for absence of all studied ten D according to gelanalyzer protocol. RAPD Analysis :A set of ten random 10-mer primers, (Table,b) was used in the detection of polymorphism among ten genotypes of D. These primers were synthesized at RAPD-PCR and carried out according to the procedure given by Williams *et al.*, (1990).

Results and Discussion

Effect of sodium azide and di-ethyl sulphate on *Borago officinalis* L. plants

On vegetative growth
First Generation (MG1)

In the first generation (MG_1) as shown in Tables (1 and 2) treating borage seeds with all treatments of sodium azide increased the plant height and stem thickness, except SA at 0.2% which decreased the plant height by 6.7%, and SA at 0.4%, which decreased the stem thickness by 13.5%. The number of branches/ plant was greatly affected by the application of SA and the highest value was recorded with SA at 0.4% with a percentage of increase that reached 42.5%. It is worthy to mention that treatment SA 0.4% has achieved the highest number of branches/plant with a percentage of increase of 42.5% The results indicated that SA enhanced formation of leaves/plant when used at 0.3 and 0.4% and the highest number (71.67) was recorded by treatment SA 0.3% with a percentage of increase that reached 12.9%. The highest percentage of decrease (51.3%) was observed for treatment of SA as compared with the control. The average leaf area in response to the application of SA , show that SA at 0.3% followed by SA at 0.2% ,were the most effective in increasing the leaf area of borage plant, the obtained increases were 81 and 60 %, respectively in comparison with the control. The fresh and dry weights of leaves were greatly affected by SA soaking treatments, as treating seeds with SA at all rates increased the fresh and dry weights of leave, with marked increases of SA at 0.3 and 0.4 %.The ratio between dry weight / fresh weight of leaves , showed evidence increase with the treatments of SA at 0.15 (0.163) and 0.3% (0.165). In Table (2), SA treatment at 0.3% increased the length of roots, whereas, the low one (0.2%) increased the No. of main roots.

Concerning the effect of diethyl sulphate (DES) as shown in Tables (1 and 2), the data indicate that all the concentrations increased the plant height and stem diameter. The tallest plants was recorded in the treatment of 0.4% DES, with 65.8% increase compared to the control. Also, DES at low dose (0.1%) increased stem diameter by 21.07 % over the control. On the other sides, treating borage with 0.4% DES gave the highest No. of branches (11.33) /plant and 0. 3% DES, produced the highest No. of leaves/ plant and highest ratio of DW / FW. The largest leaf area was recorded with o.2% DES. The fresh and dry weights of leaves were the highest with low DES dose . All DES doses increased the root length as compared with control ,the longest roots and the highest No. of main roots / plant were obtained in plants produced from 0. 4% DES, in MG1. The comparison between the effect SA vs DES , revealed that DES at 0,3% showed higher mutagen efficiency on most traits of vegetative growth than SA and control treatments. However, it is worth to mention that the treatment of SA at 0.3% showed a remarkable increase in the formation of leaves /plant, average leaf area, fresh and dry weights of leaves, he percentage of dry weight relative to fresh one.

Second Generation (MG2)

In MG2, the results (Tables(1 and 2) indicate that soaking borage seeds in SA at 0.3 %, significantly increased the plant height ,No. of leaves, fresh and dry weights of leaves as well the ratio of dry weight to fresh one, in comparison with other doses of SA.But,stem thickness responded well to SA at 0.1 % . The No.of main branches/ plant was greatly affected by SA at 0.4 %.. There was a reduction in No.of leaves formed on the plants when seeds were soaked in SA at 0.1, 0.2 and 0.4%, as compared with the control. But, there was unexpected increase in the formation of leaves, when seeds was soaked in SA at 0.3%. The ratio between DW/FW of leaves showed a marked increase with the low dose of SA at 0.1 as well as 0.3%%. All SA treatments decreased the No. of main roots and root length, in comparison with the control , ith the exception of SA at 0.3%, which increased the length of roots to 31.57 cm with increase 53.78 % over the control.

Table 1. Effect SA and DES on plant height, stem diameter, No. of branches/plant and No.of leaves/plant,fresh, dry weights of leaves(g) and leaves DW /FW ratios of Borago officinalis L. during the season of 2019/2020 and 2020/2021.

Mutagens	Plant height (cm)	Stem Diameter (cm)	No. of branches	N0.of leaves	Leaf area (cm^2)	FW of leaves (g)	DW of leaves (g)	DW /FW ratios
	M1 (First generation)							
Control	53.37	2.23	7.67	63.50	26.6	129.03	19.82	0.153
SA 0.1 %	57.10	2.70	10.87	58.50	27.8	131.40	21.45	0.163
SA 0.2 %	49.77	2.40	9.75	55.67	44.6	140.18	21.98	0.157
SA 0.3 %	59.20	2.53	10.00	71.67	48.0	154.05	25.40	0.165
SA 0.4 %	63.73	1.93	11.33	64.83	31.0	145.61	22.12	0.152
DES 0.1 %	71.07	2.30	9.67	63.00	61.0	173.62	29.87	0.172
DES 0.2 %	74.17	2.57	9.75	73.67	68.4	166.40	27.44	0.165
DES 0.3 %	75.10	3.20	11.33	80.33	48.4	152.37	26.92	0.177
DES 0.4 %	88.50	2.43	7.75	48.67	48.0	146.98	24.45	0.166
LSD 5%	6.28	1.18	1.56	5.28	4.84	15.12	2.97	-----
	M2 (Second generation)							
Control	61.67	2.30	10.74	64.33	26.2	124.49	19.45	0.156
SA 0.1 %	54.50	2.53	11.88	61.60	32.2	126.09	21.23	0.168
SA 0.2 %	47.67	2.33	10.50	52.67	51.0	148.51	22.97	0.155
SA 0.3 %	69.33	1.90	10.33	77.60	42.8	190.26	31.00	0.163
SA 0.4 %	61.00	1.90	12.67	62.83	44.2	157.56	24.64	0.156
DES 0.1 %	64.67	2.43	10.00	61.13	53.0	180.62	27.84	0.154
DES 0.2 %	69.00	2.57	10.50	75.73	43.2	186.58	35.28	0.189
DES 0.3 %	75.67	2.97	12.00	76.60	37.4	162.51	27.21	0.167
DES 0.4 %	43.50	3.03	10.75	86.53	35.2	160.23	23.42	0.146
LSD 5%	6.43	0.81	1.65	6.81	3.5	13.03	2.11	------

The response of different growth parameters to the different doses of DES in MG2 (Tables 1 and 2), indicate that 0.1,0.2 and 0.3% doses increased the plant

height which was the tallest in plants treated with 0. 3%, whereas raising the level to0. 4% significantly decreased it, but, this dose gave the thickest stems .On the other sides, treating borage seeds with 0.3% DES produced the highest No. of branches and DES at 0.2 -0.4% increased the formation of leaves/ plant , giving the highest number with the highest dose 0.4% ,with 34.51% increase compared to control.

Table 2. Effect of sodium azide and di-ethyl sulphate on plant height, stem diameter, No. of branches/plant and No. of leaves/plant, fresh and dry weights of leaves (g) of Borago officinalis L. during the season of 2019/2020 and 2020/2021

Mutagens	Root length (cm)	No. of main roots	Day to Floweri n	florets/ plant (no.)	Fruit set (%)	Seeds yield /plant (g)	No. of seeds /g	Seed/f ed (Kg)
	M1 (First generation)							
Control	20.53	8.36	147.90	209.80	77.33	10.87	67.23	260.88
SA 0.1 %	19.70	7.53	151.33	187.60	76.67	11.84	61.30	284.16
SA 0.2 %	18.53	8.17	145.33	216.60	83.50	13.51	63.77	324.24
SA 0.3 %	31.57	6.20	143.50	199.40	75.87	12.12	64.22	290.88
SA 0.4 %	19.47	7.07	138.00	222.07	64.16	11.79	59.85	282.96
DES 0.1 %	20.75	6.67	155.67	253.40	80.50	13.11	63.42	314.64
DES 0.2 %	23.05	8.72	147.83	212.37	82.50	12.60	65.06	302.40
DES 0.3 %	21.55	7.27	144.33	191.90	68.33	11.56	60.67	277.44
DES 0.4 %	28.40	10.50	132.00	126.90	65.40	10.58	58.44	253.92
LSD 5%	3.10	1.78	7.44	10.32	5.58	1.56	5.22	--
	M2 (Second generation)							
Control	20.23	7.57	153.3	214.43	79.44	7.15	68.55	171.06
SA 0.1 %	18.90	5.63	147.0	198.93	78.25	7.28	64.36	174.72
SA 0.2 %	17.75	6.53	144.5	228.33	77.63	8.47	62.20	203.28
SA 0.3 %	20.13	7.07	140.5	177.73	69.69	6.19	59.22	148.56
SA 0.4 %	26.20	8.03	141.3	205.20	70.56	7.46	59.55	179.04
DES 0.1 %	2613	7.53	155.7	241.33	74.58	9.95	61.42	238.08
DES 0.2 %	22.65	7.10	152.5	193.83	84.73	8.25	67.06	198.00
DES 0.3 %	21.15	7.23	148.3	183.87	82.55	7.83	63.60	187.92
DES 0.4 %	27.15	6.53	144.0	169.13	71.32	7.04	66.25	168.96
LSD 5%	7.98	2.32	5.60	8.42	6.33	2.45	6.00	-

The largest leaf area in plants treated with DES was recorded at low dose 0.1%, and the heaviest FW and DW of leaves were obtained with 0.2% dose , giving also the highest value of DW / FW ratio . All DES doses increased the root length as compared with control ,the longest roots and the highest No. of main roots were recorded in plants produced from the 0. 4% DES. The comparison between the two chemical mutagens (SA vs DES), revealed that borage seeds treated with 0.3% DES showed higher positive mutagen effects on most traits of VGP than SA and control. whereas 0.4% SA was more effective than the other doses of SA and the control in enhancing plant height and branching and the treatment of SA at 0.3% showed a remarkable increase in formation of leaves, leaf area, FW &DW of leaves than the other doses of SA

Leaf morphological changes

In comparison with the control ,the different doses of SA and DES induced various of leaf morphological changes in leaf size, shape, margin and petioles (Fig.1)

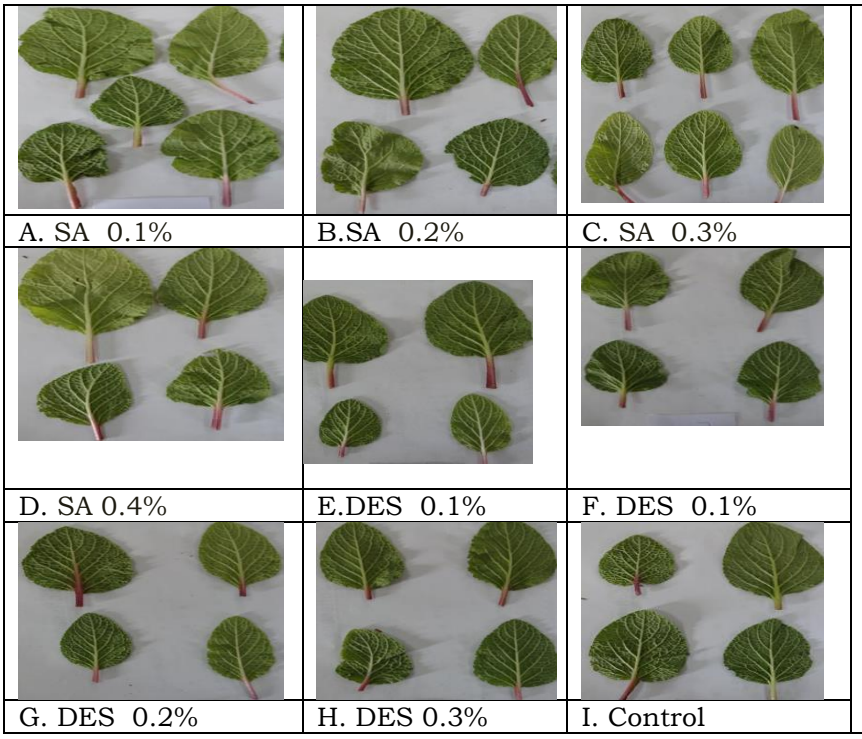

Fig (1): Changes i n leaf size, shape, margin , length and thickness of petiole were detected in most treatments of SA and DES in M2generation as follows :
A- SA 0.1%
B- SA 0.2%
C- SA 0.3%
D- SA 0.4%
E- DES 0.1%
F- DES 0.2%
G- DES 0.3%
H- DES 0.4%
In comparison with the control.

On Flowering and seed production

As shown in Table (2) and Fig.(2), in the MG1, the application of SA and DES at all concentrations on borage plant showed a great effect on flowering of borage plant. The control plants began flowering after 148.50 days after sowing the seeds, whereas soaking seeds in the low level of both SA (0.1%) and DES (0.1%) , prolonged the vegetative growth phase(VGP),whereas others doses markedly shortened the VGP ,enhanced flowering stage and the highest dose was the most effective in this respect. The application of SA at 0.4 % and DES at 0.1 were the most effective in increasing the No. of florets/plant. The revealed that SA at 0.2 % and DES at 0.1 and 0.2% were the most effective in increasing the fruit set%, which in turn increase the yield of seeds / plant, which may reach a maximum of four in borage flowe. The higher doses of SA(0.4 %) and 0.3and 0.4% % of DES showed higher rates of fruit set failure, which markedly decrease the seed yields and economic returns than the control and the lower doses of these mutagens. The seeds yield/ plant was correlated to the frit set%, so the high values of this trait was obtained with SA0.2 % and DES 0.1 and 0.2% .The No. of seeds/g, was between 58.44 for 0.4% DES to 67.23/g for the control.

In MG2, SA and DES at all doses (expect 0.1% DES) greatly shortened the VGP and enhanced flowering stage (days to first flower),and reduced the vegetative growth phase(VGP), which reached to 140.5with 0.1% SA, as the control plants began flowering after 153.3days after planting seeds. The longest VGP (155.7days) was recorded in 0.1% DES followed by 0.2%.The treatments of SA at 0.2 % and DES at 0.1% significantly increased the formation of florets per plant, giving 228.33 and 241.33 florets /plant, against 214.43 florets for the control. The percentage of friut set was found to increase uniformly with increase in doses of the mutagens used, as the application of DES at 0.2% and 0.3% had more profound influence in increasing the fruit set%.Plants from seed-soaking of 0.1% DES and SA 0.2% produced the highest values of seed yield (g) /plant (9.95 and 8.47 g , respectively),as well as the production of seeds (kg) per fed.

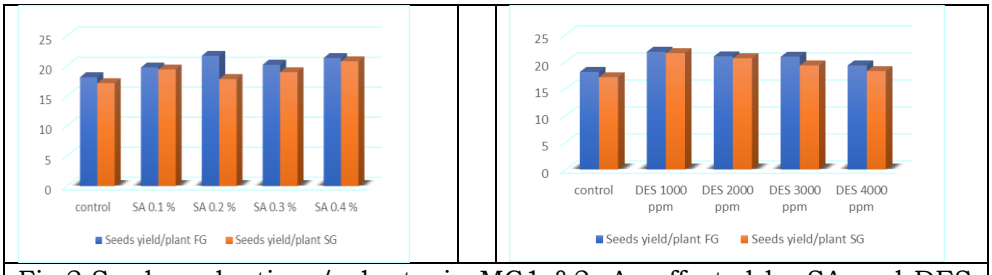

Fig.2.Seed production / plant , in MG1 &2, As affected by SA and DES at different doses.

On chemical composition

In the MG1, as shown in Table (3) most of SA and DES treatments increased the contents of chlorophyll-a,-b than the control plants. There was a marked reduction in carotenoids content, with SA treatment, whereas DES at 0.2% and 0.3% increased it. In the MG2, the data indicated that (Table, 3) most of SA and DES treatments increased the contents of chlorophyll-a,-b and carotenoids. The content of total carbohydrates was markedly decreased with SA (except SA at 0.3%), whereas all concentrations of DES increased it. In MG2 (Table,3) the content of total carbohydrates in leaves, showed a similar trend as in the first generation (MG1).

Table 3. Effect of sodium azide and di-ethyl sulphate on plant height, stem diameter, No. of branches/plant and No. of leaves/plant, fresh and dry weights of leaves (g) of Borago officinalis L. during the season of 2019/2020 and 2020/2021

Mutagens	Chl.-a (mg/g)	Chl. – b(mg/g)	Carotenoids (mg/g)	Carbohydrates % (DW)	Fixed oil %	Oil yield / plant (g)	Oil yield / fed (kg)
	M1 (First generation)						
Control	2.90	1.47	1.37	19.65	19.66	2.137	51.289
SA 0.1 %	3.41	1.86	0.96	17.75	24.82	2.938	70.528
SA 0.2 %	2.80	1.49	1.05	18.30	24.36	3.291	78.984
SA 0.3 %	4.02	2.61	1.25	20.05	24.46	2.964	71.149
SA 0.4 %	3.04	1.63	1.20	17.10	25.50	3.006	72.154

DES 0.1 %	2.19	1.93	1.09	22.05	27.32	3.581	85.959
DES 0.2 %	4.47	1.66	1.55	22.60	25.38	3.197	76.749
DES 0.3 %	3.88	1.90	1.49	23.00	26.22	3.031	72.744
DES 0.4 %	3.40	1.76	1.24	23.07	23.08	2.441	58.604
LSD 5%	0.45	0.25	0.33	1.85	3.55	0.14	0.45
	M2 (Second generation)						
Control	2.68	1.55	1.05	20.74	22.82	1.631	39.159
SA 0.1 %	3.48	1.80	1.40	19.86	23.86	1.737	41.688
SA 0.2 %	2.84	1.28	1.21	19.91	24.48	2.073	49.762
SA 0.3 %	3.17	1.51	1.47	14.03	25.54	1.580	37.942
SA 0.4 %	3.96	1.40	1.51	17.77	25.10	1.872	44.939
DES 0.1 %	4.34	1.79	1.35	20.80	24.58	2.445	58.697
DES 0.2 %	2.77	1.94	1.22	22.97	26.06	2.149	51.598
DES 0.3 %	3.12	1.61	1.36	23.31	18.52	1.450	34.802
DES 0.4 %	3.00	1.34	1.26	23.35	16.44	1.157	27.777
LSD 5 %	0.40	0.27	0.42	1.70	2.68	0.21	-

Concerning the responses of oil content(fixed oil) in the MG1 to the treatments of SA and DES(Table3 and Fig.3),it was found that all treatments of SA and DES increased it, but in the second generation (MG2) the contents of chlorophyll-a,-b than the control plants. There was a marked reduction in carotenoids content, with SA treatment, whereas 0.2 and 0.3% % DES doses increased it. In the MG2, the data indicated that (Table3) most of SA and DES treatments increased the contents of chlorophyll-a,-b and carotenoids. The content of fixed oil in seeds, was markedly decreased with DES at 3000 and 4000 ppm, the other concentration of both SA and DES increased it. The highest oil content in MG1 (27.32 %) and in MG2 (26.06%) was recorded in plants from DES at 0.1 and 0.2% respectively. There was a marked reduction, when the level of DES increased to 0.3% or 0.4%, in the MG2 only. The highest value of fixed oil yield (g) /plant and feddan, in MG1 (3.581g/plant , 85.959kg/ fed.) and in MG2(2.445 g and, 58.697kg),were recorded with DES at 0.1%.

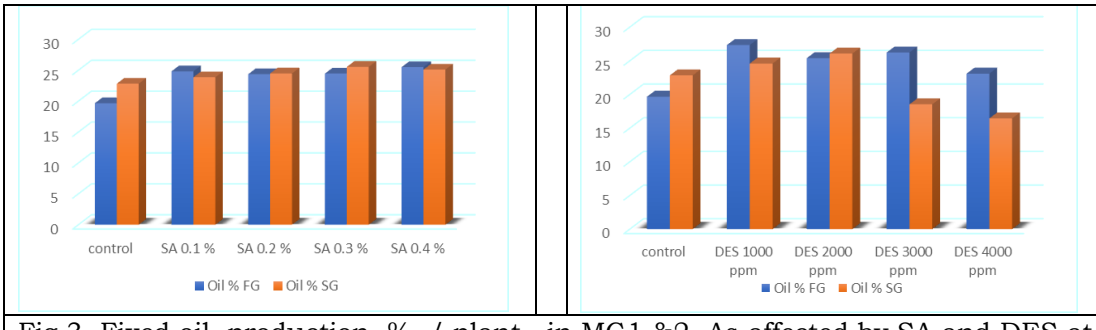

Fig.3. Fixed oil production % / plant , in MG1 &2, As affected by SA and DES at different doses.

Genetic diversity and distance

They can be identified by using morphological, biochemical, and molecular markers. Morphological traits can be divided into qualitative and quantitative

traits and the best time for the identification of mutant plants is the M2generation. In the M2 generation, mutations that possibly occurred in the M1generation segregate to create homozygotes recessive and/or dominant alleles.

RAPD assay

using five mutagen namley; SA 0.2 %; SA 0.4%; DES 0.1% ,0.3% and 0.4% (Table.4 , 5 and Fig.4). Using ten RAPD- PCR technique revealed ninety one variables bands, were of them 61 polymorphic bands, with unique bands by 67% of polymorphism and 30 of them monomorphic bands under effect five mutants SA 0.2 %; SA 0.4%; DES0. 0.1% ,0.3% and 0.4%.

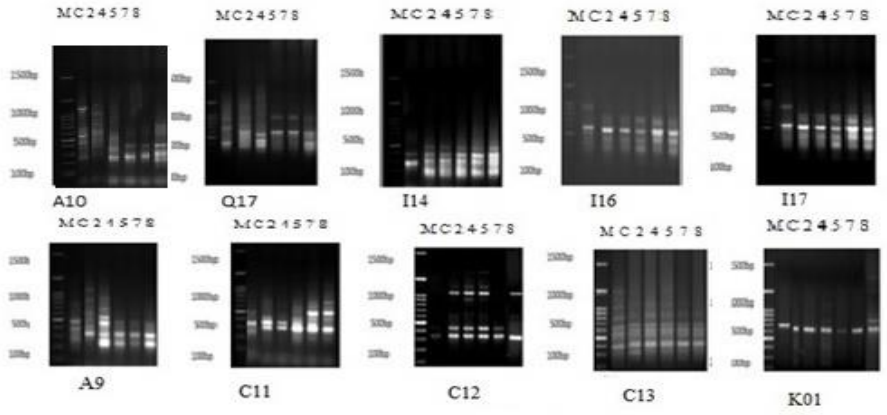

Fig 4: DNA banding patterns using RAPD-PCR under effect five mutants.

Genetic relationship was divided into two parts, the first one of which included 3 genetic genotypes (control, DES 0.4% and SA 0.4 %, SA 0.2% respectively.The second cluster includes DES 0.1% only.

Table 4. Polymorphic, monomorphic, unique bands , polymorphism and range of bands (bp) for ten RAPD primers

Primers code	Polymorphic bands	Monomorphic bands	Unique bands	Total bands	Poly-morphism %	Range of bands (bp)
A10	0	3	3	8	50	190:855
Q 17	4	4	3	11	70	424:1210
I 14	1	6	3	10	40	243:1612
I 16	3	3	3	9	66.7	140:640
I 17	6	5	0	11	54.5	170:1255
A9	4	1	1	6	83.3	210:1230
C 11	3	2	3	8	62.5	180:1190
C 12	4	1	4	9	55.6	350:1320
C 13	5	2	3	10	80	160:1200
K 01	4	3	4	11	77.7	130:1050
Total	34	30	27	91	67%	105:1612

Table 5: Proximity matrix using SPSS version 20

Case	Control	SA at 0.2%	SA at 0.4%	DES 0.1%	DES 0.3%	DES 0.4%
Control	1.000					
SA at 0.2%	.739	1.000				
SA at 0.4%	.874	.577	1.000			
DES 0.1%	.566	.261	.257	1.000		
DES 0.3%	.668	.900	.719	.034	1.000	
DES 0.4 %	.890	.500	.758	.588	.479	1.000

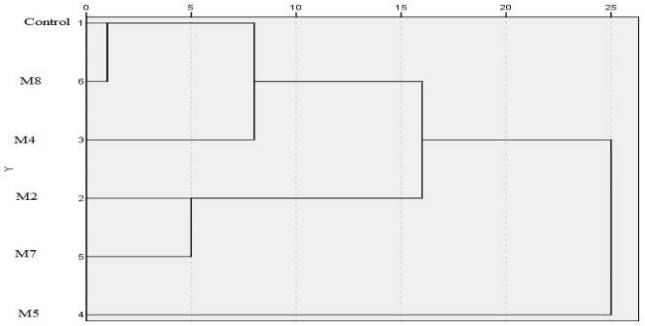

Fig.5. Dendrogram of the genetic distances among the genotypes based on RAPD analysis under effect five mutants of borage plant

Discussion

In MG1, most of SA doses increased plant height and stem thickness, ramification, leaves formation, leaf area, FW and DW of leaves, DW /FW ratios and root length, which can be attributed its effect on the meristematic tissues of the seeds, besides the physiological and acute chromosomal damage as reported by (Nilan *et al.,* 1976 and Singh *et al.,* 1997) or to the ability to enter the cells to interact with the DNA and protein synthesis (Mostafa 2011). SA 0.2% increased the roots. In MG2, SA at 0.2 % increased most morphological traits, and stem thickness responded well to SA at 0.1%. The leaves DW/FW ratios, showed a positive response to SA 0.1 and 0.3%. DES 0.1%, gave thickest stem and DES 0.3 % improved the formation of leaves and at 0.2% gave the largest leaves. In MG2, DES at 0.3%improved the ramification of branches, at 0.2%increased No. of leaves and leaf area. The low dose 0.1% resulted the heaviest weights of leaves.SA at 0.2% and DES at 0.4% markedly increased the roots growth. The comparison between the effect of different doses of SA vs corresponding doses of DES, indicated that DES at 0.2- 0.3 % showed higher mutagen efficiency on most vegetative growth traits. (Wannajindaporn 2016) on Dendrobium obtained morphological differentiation in some putative mutants: reduced height, shorter and thicker leaves, and shorter and fewer roots, with SA treatment compared with controls. The less effect of SA on morphological alterations may result from either genetic changes as a result of mutation or physiological changes due to using higher doses of NaN_3, which showed a tendency to induce multiple morphological alterations, alterations in the physiological and biological processes included or necessary for vegetative growth. In this regard, (Kleinhofs *et al,*1978) reported

that azide ions in the solution play an important role in causing the mutations by interacting with enzyme and DNA in the plant cells, or as inhibitors of cytochrome oxidase, causing inhibition in the oxidative phosphorylation process which alters the mitochondrial membrane potential. Whereas, (Al- Qurainy and Khan 2009), they stated that the effect of SA may hamper ATP biosynthesis resulting in decreased ATP molecules. The plant cells require ATP molecules in the all biological reactions. The variation in days to flowering induced by SA, may be due to damage in cell constituents at molecular level or altered enzyme activity (Ali *etal;* 2014). The poor seed set due to SA may be attributed to the effect of in producing defective pollen grains and temperature prevailing during and after blooming, in addition to the long flowering period (from March to May), as there were various hormonal shifts. Similarly, (Roychowdhury and Tah 2011) observed that SA treated at 107.66 mM made changes in pollen sterility and agro-metrical traits, which may result from either genetic changes as a result of mutation or physiological changes. While, (Kamra and Brunner 1970) stated that the acidic products of DES and EMS could be controlled by buffering the mutagenic solutions (pH, buffer type, molarity of the buffer, photolysis) may produce different effects. The dose of 0.3%DES various increases in morpholocal and flowering traits as well seed and oil production, which may be due to the physiological stimulation as reported by (El-Torky 1992) or may be attributed to increase in the rate of cell division rates, cell elongation, and activation of some hormones(auxin) as reported by (Joshi *et al.,* 2011 and Mostafa *et al.,* 2014). In MG1 Chl. a increased with SA at 0.3%and DES 0.2 % the, Chl. b recorded the lowest value by SA0.1%, whereas carotenoids increased in SA 0.4% and DES at 0.2%and 0.3%, all SA reduced carbohydrates content. In MG2, all SA &DES increased the content of Chl-a (except DES 0.2% and carotenoids (except DES 0.2%). In case of Chl. b, SA at 0.1 and 0.3% increased it. The lowest carbohydrates recorded in SA at 0.2%. The fixed oil content of seeds in MG1 recorded the highest value with DES 0.2%and with DES 0.4%in MG2. According to (Animasaun and Oyem 2014) applying SA at the optimal dose induces an increase in the content of proteins and fats in seeds. SA at the low doses may induce enzyme activity such as catalase and lipase and hormonal activity and it may be attributed in increasing growth promoters, gave sudden increase in metabolites of seeds at certain dose, increase in destruction of inhibitors, drop in the auxin level or inhibition of auxin synthesis and decline of assimilation mechanism. Using ten RAPD- PCR technique reveled ninety one variables bands, 61 polymorphic bands, with unique bands by 67% of polymorphism and 30 of them monomorphic bands.

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