Effect of Gamma rays, Microwave and Colchicine on Some Morphological and Cytological Characteristics of *Gladiolus grandiflorus* c v. White Prosperity.

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ABSTRACT

Two field experiments were carried out at the Experimental Farm of Horticulture Department, Faculty of Agricultuer. Moshtohor, Benha Univeristy, Egypt in the two successive seasons of 2015/2016 and 2016/2017 to evaluate the effect of physical mutagen (Gamma rays and Microwave) and chemical mutagen (colchicine) on *Gladiolus grandiflorus* cv. 'White Prosperity '. and *Antholyza ethiopica* The corms were treated by Gamma rays at. 20 Gy, 40 Gy, and 80 Gy, different concentrations of colchicine (0.1 and 0.2% for 20 hours) and different times of microwave (10, 20 and 30 seconds) Growth performance, flowering, corm production and mutations production of *Gladiolus grandiflorus* c v. White Prosperity and *Antholyza ethiopica*. Results showed that the different levels of colchicine had a slight effect on sprouting date, but Gamma rays and microwave treatments induced a significant earliness in both seasons.

All treatments showed significant increased the studied growth parameters, i.e. plant height, number of leaves/plant, length at the fourth leaf (cm), leaf area at the fourth leaf (cm2) and fresh and dry weight of leaves (g)/plant as compared with control. The best results were obtained by microwave at 10 second treatment and gamma rays at 20 Gray. The greatest treatment was recorded in the two seasons by soaking the corms in 0.2% colchicine solution for 20 hours in both seasons.

However, first opening flower was affected by all tested treatments except colchicine at 0.1% and 0.2% and microwave treatment at 30 second. Also, flower spike fresh weight, florets number per spike, flowering zone length, flower spike length in both seasons were affected.

Conclusively, the study recommended to soak the corms in 0.1% and 0.2% colchicine solution for 20 hours. Exposed to the corms to gamma rays and microwave before planting to get better growth performance. All parameters the exposure of gladiolus corms to higher doses of gamma rays at 80 Gray and microwave at 30 second were significantly decreased all parameters under this study.

Additionally, maximized lagging chromosomes stickiness and polypolar cells of chromosomal abnormalities were show at Subjecting of gladiolus for microwave for 10 seconds and gamma rays at 40 and 80 Gray, succeeded in inducing low chromosomal abnormalities, stickiness aneuploidy, polyploidy, and micronucleate per cells. immersion of gladiolus corms in 0.02% diploid chromosomes, hexaploidy, lagging chromosomes, stickiness aneuploidy, polyploidy and micronucleate per cells.

Key words: *Gladiolus grandiflorus*, Radiation, Gamma rays, Microwave, Colchicine, Vegetative Growth, Plant, Flowering, Corms, Bulbus, Mutation, chromosomes, Diploidy, Hexaploidy, Morphological, Cytological.

Introduction

Gladiolus grandiflorus belongs to family *Iridaceae*. It is a bulbous flowering perennial which is Commercially cultivated as cut flower throughout the world. due to its excellent vase life and captivating colors, it has great economic value in global trade in landscaping **(Singh and Sisoda, 2017).** Gladiolus is represented by 180 species and 10000 cultivars including almost all colors. Many cultivars varied in size, color, flowering date and other flowering aspects such as White Prosperity, Eurovision, Novolux, Rose Supreme, Peter Pears, Sancerre and others have been recently introduced to Egypt. Planted areas with such Gladiolus cultivars in Egypt is much expanded in order to meet the increase demand for Gladiolus flowers for local market and exportation.

In this study corms of Gladiolus cultivar White Prosperity were chosen for its popularity and adaptability to the Egyptian environmental conditions. Also, White Prosperity has some important characters such as its favorable height (80 to 100 cm), sturdiness of stem is good with large florets size which is showy florets (7.0 to 8.0 cm) **(Hogan, 1990).** Breeders have been resorting to generate variation through artificial hybridization and/or mutation breeding in many ornamental crop and these methods have served the breeders effectively.

Most of the wild species have diploid chromosome numbers based on x = 15, in contrast with modern cultivars of *G. grandiflora* which are tetraploid, Doubling the chromosome number of wild species (diploid, 2n = 2x = 30) to match modern cultivars (tetraploid, 2n = 4x = 60) is an important procedure for interspecific hybridization breeding. Gamma rays (is a part of electromagnetic spectrum) belong to ionizing radiation, the biological effect of gamma rays is based on the interaction with atoms or molecules in the cell, particularly with water to produce free radicals in cells. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the irradiation level. These effects include changes in the plant cellular structure and metabolism, dilation of thylakoid membranes alteration in photosynthesis, modulation of the antioxidative system and accumulation of phenolic compounds. **(Hamideldin and Hussin 2014).** Colchicine is an alkaloid obtained from the root of *Colchicum autumnale L.*, a wild plant native to the Mediterranean region. The chemical formula of purified colchicine is C22H25NO6. Colchicine is one of chemical commonly to induce polyploid in plants with high success rates. In addition to the number of different chromosomes, polyploid plants are characterized by different morphology compared to diploid plants.

Microwaves are nonionizing radiations which are the part of the electromagnetic spectrum. **[Ragha, *et al.*, 2011].** It also affects the cell growth rate as well as interaction with ions and organic molecules. A few studies have demonstrated that microwave radiation has a positive effect in accelerating seed sprouting **(Chen *et al., 2005*).** Therefore, the purpose of this study was to evaluate the effect of physical mutagens (gamma rays, microwave) and colchicine on morphological and cytological Characteristics, vegetative growth, flowering and corm production.

Materials and Methods

This investigation was carried out during the two successive seasons of 2015/2016 and 2016/2017, at the Experimental station of the Faculty of Agriculture, at Moshtohor, Benha University.

I- Plants materials:-

Apparently healthy corms 9.95 gm mean fresh weight and 2.7to 3 cm in diameter of *Gladiolus grandiflorus* importing from Netherlands, were planting on 15th September in two seasons. As for field part the rows of the field were ridged at 50 cm apart and the corms were planted at 20 cm apart with depth of 10 cm. each treatment contained three replication, each replication content 10 corms. All corms which planting in three treatments were soaked in Topsin as (fungicide) at the concentration of 1 g/l. for one minute**, (Ghatas,2016).** then washed with tap water and dried befor Planting immediately. Physical and Chemical properties of the soil used were analyzed at Lab. of Soil and Water, Dept., Moshtohor Fac. of Agric, Benha Univ. are presented, in Tables A and B**.**

Table (a). The physical properties of the soil.

Texture	Sand%		Clay%	Silt%
	Fine	Coorse		
Clay	5.60	7.44	53.57	33.50

Chemical properties of experimental soil soluble actions and anions mMol/l available (ppm).

Table (b). Soil chemical properties of the soil(mMol/l) available (ppm).

pH	E. CDs/m	So_4^{--}	Cl^-	HCo_3^-	Mg^{++}	Ca++	K^+	Na^+	N	P	K	Fe	Mn	Zn
7.36	0.62	0.80	3.4	2.00	1.20	2.50	1.23	1.27	918.7	17.8	431.7	941.1	450.1	72.43

Layout of the experimental:

The experimental was in a complete randomized block design with three replicates each replicate contain 10 plants.

Treatments:

A- <u>Gamma ray treatments:</u>

Dry corms of the two plants were irradiated before planting in farm using gamma-cell. using Gamma cell 40/Date (April-77) –curies (3032)- (cesium-137) source from a unit gamma chamber at dose rate of .713 .0 rad / second .at the National Center for Radiation Research. and Technology, Nasr City, Cairo, Egypt (cesium-137). Irradiation doses were

0,0,20,40,80Gray (1Gy =100 rad). then the corms were planting on 15 /9/2016 and 2017 for the two seasons. **Khalifa (2016).**

B- Colchicine treatments:

Colchicine ($C_{22}H_{25}NO_6$) an alkaloid product, obtained from El- Gomhoria Company, Alexandaria branch. Corms of both plants were dipped in different concentrations at 0.1% and 0.2% for 20 hours. While, control corms 0.0% of treatment were soaked in tap water for 20 hours.

C-Electromagnetic waves:

Corms of two kind were exposed to 390 waves from microwave for 10, 20, or 30 seconds. The microwave used in this treatment was single phase with grounding 1.3 K.W. output 650 at a frequency of 2450 MH2 (model # Mo6t,22 v-soH2) at Lab. of microbiology, Dept., Moshtohor, Faculty of Agriculture, Benha University. The normal agricultural practices (irrigation, ………e t c) were carried out for the experiment.

Studied character:

1. sprouting:

Number of days required for sprouting and Corm sprouting percentage.

2. The vegetative growth:

Vegetative growth characters just before flowering were recorded: Plant height (cm), Leaves area at the 4^{th} leaf (cm2), Number of leaves /plant, Leaves fresh and dry weight (g).

3. Flowering growth:

Flowering date, The length of spike, Flowering zone length, Number of florets per spike, Fresh and dry weights of flower spike, Vase life.

4. Corms and cormels production characters:

Number of corms / plant, Diameter of the corm (cm), Fresh weight of corms / plant (g), Fresh weight of cormels /plant (g), Number of cormels / plant.

5. Cytological studies:

The cytological studies were carried out on the root tips of Gladiolus plant which has 30 chromosomes in cytological laboratory, Genetic Department, faculty of agriculture at moshtohor, Benha university. And using the method according to **(Dhaduk, 1992).**

Statistical analysis: All obtained data in both seasons of study were subjected to analysis of variance as factorial experiments in a complete randomize block design. L.S.D. method was used to differentiate between means according to **Snedecor and Cochran (1989).**

RESULTS AND DISCUSSION

1. Number of days required sprouting:

Table (1) show that gamma rays and microwave irradiation at doses 20 Gray and microwave at 10 second highly significantly increased Number of days required sprouting in the two seasons(early sprouting) comparing with the control treatment. the maximum increase was achieved with gamma rays 20 Gray. followed by microwave at 10 second treatment gave the next results, but gamma rays at 40 Gray produced the third value in this concern in both season. Whereas, gamma rays at 80 Gy, colchicine at 0.1%,0.2% microwave at 20,30 second caused a significant decrease Number of days required sprouting in both season. In both seasons treating *Gladiolus grandifloras* cv white Prosperity with gamma rays at dose 20 gray appearing to be the most effective compound for formatting the maximum number of days required sprouting when compared with other treatments and control. The differences between all treatment were significant at 0.05 in both seasons. Agreed with those obtained by **Patil (2017), Patel *et al*,. (2018).** on Gladiolus. It was observed that there was negative correlation of sprouting and survival per cent with the different treatments.

2- sprouting percentage %:

Table (1) show that gamma rays irradiation at doses 20 and 40 Gray, colchicine at 0.1 and 0.2% and control treatment highly significantly increased sprouting percentage % (100%) in the two seasons. followed by gamma rays at 80 Gray treatment gave the next results, but microwave at 10 and 20 second produced the third value in this concern, in both season. Whereas, microwave at 30 second caused a significant decrease sprouting percentage % in both season. In both seasons treating *Gladiolus grandifloras* cv white Prosperity with gamma rays at dose 20 gray appearing to be the most effective compound for formatting the maximum sprouting percentage % when compared with other treatments. The differences between all treatment were significant at 0.05 in both seasons.

his early sprouting of gladiolus corms by lower dose of gamma rays was probably related with the increase in the activities of gibberellins and auxins and disappearance of inhibitors**.** It is also known that amylolytic activity is shown by germinating corms due to pretreatment with gamma rays before soaking them for sprouting. It was shown that with changing gamma rays, the gene expression, protein biosynthesis, enzyme activity, cell reproduction and other activities of cellular metabolism increases in the plant.

This early sprouting of gladiolus corms at lower dose of gamma rays was probably related with the increase in the activities of gibberellins and auxins and disappearance of inhibitors**. Karki and Srivastava (2010).**

These results are agree with **(Kuldeep *et al*., and Sudha, 2016)** on *(Gladiolus hybridus).* **And Patel *et al* ,. (2018).** on Gladiolus. who found that the increase in sprouting percentage % and stimulating number of days required sprouting may be due to physical treatments action on enhancing the hydrolysis of the complex compounds (carbohydrate, fats, proteins, …….etc) in corms to simple compounds (sugar, fatty acid, amino acid,..….etc) this could happen directly or indirectly by affecting the enzymatic reactions in corms**.**

Table, (1): Effect of Gamma rays, Colchicine and Microwave on sprouting of *Gladiolus grandiflorus* plant during two seasons 2015/2016 and 2016/2017.

Character		No of days required sprouting		sprouting percentage	
Treatment		1st season.	2nd season.	1st season.	2nd season.
Control		19.00	18.30	100.00	100.00
Gamma rays	20Gy	12.00	10.33	100.00	100.00
	40Gy	16.00	13.66	100.00	100.00
	80Gy	18.00	17.66	88.88	86.00
	Mean	14.33	13.89	96.29	95.33
Colchicine	0.1%	19.00	19.33	100.00	100.00
	0.2%	18.66	18.90	100.00	100.00
	Mean	13.67	14.44	100.00	100.00
Microwave	10 second	12.33	11.33	50.00	55.00
	20 second	17.00	16.00	50.00	54.00
	30 second	21.00	19.33	33.33	35.00
	Mean	17.33	15.56	44.44	48.00
L.S.D. at	.05	2.210	2.064	2.55	2.42

1. The vegetative growth:

Data in Table (2) showed that the corms of Gladiolus treated by three different material as Gamma rays at (20, 40 and 80 Gy), Colchicine at (0.1 and 0.2%) and Microwave at (10 second, 20 second and 30 second) All treatments except gamma rays at 80 Gy, microwave at 30 second. significant increase of the studied growth parameters, (plant height, number of leaves/plant, length of the fourth leaf (cm), leaf area at the fourth leaf (cm2), fresh and dry weight of leaves (g)/plant) over control, but the best treatments were obtained by Microwave at 10 second treatments and Gamma rays at 20 Gray. The greatest treatments were recorded in the two seasons by soaking the corms in 0.2% colchicine solution for 20 hours in both seasons.

Reduction of growth, especially at higher concentrations or soaking for long duration may be attributed to the high toxicity of the used mutagens and its direct role on depression the biosynthesis of some amino acids, such as L-cysteine and O-acetyl serine in the meristematic cells whereas increasing mean number of leaves may refer to the ability of these mutagens to overcome the apical dominance and hence, stimulate the lateral buds to develop into new leafy shoots. These findings however are in accordance with those observed by **Xing *et al.* (2011)** on *Catharanthus roseus* and **Wand and Lei (2012)** on *Clivia miniata,* The stimulative effect of low doses of gamma rays irradiation on growth may be due to the increase of cell length or cell number and size shifting in metabolism which promoted the stimulating effect of photohormones on biosynthesis of nucleic acids. It has been suggested that Gamma rays treatment also 27 activates enzyme activity and protein formation, cell reproduction, photochemical activity, respiration rate, and nucleic acid content**.**

Low level of mutagen itself is not responsible for vegetative growth of gladiolus but it influences the activity of enzymes. Enzymes play an important role in plant metabolism to accelerate metabolism activities and consequently result in stimulating plant growth **,** These results are agree with those reported by **Patil *et al.*, (2010)** on *(Gladiolus hybridus),* **Xing *et al.* (2011)** on *Catharanthus roseus* and **Wang and Lei (2012)** on *Clivia miniata.*, **Zhari, (2016) and Sudha, (2016), (Kuldeep *et al*., (2017)** on *(Gladiolus hybridus)***.** The stimulative effect of low doses of gamma rays irradiation on growth may be due to the increase of cell length or cell number and size shifting in metabolism which promoted the stimulating effect of photohormones on biosynthesis of nucleic acids. It has been suggested that Gamma rays treatment also 27 activates enzyme activity and protein formation, cell reproduction, photochemical activity, respiration rate, and nucleic acid content**. Patel *et al,*. (2018).** on *(Gladiolus hybridus).*

Table, (2) Effect of Gamma rays, Colchicine and Microwave on Vegetative growth of *Gladiolus grandiflorus* plant during two seasons 2015/2016 and 2016/2017.

Character		plant height (Cm)		No of leaves /plant		length of the 4th leaf cm		Area of the 4th leaf (cm)		Leaves fresh weight (g)/plant		Leaves dry weight(g)/plant	
seasons Treatment		1st s.	2nd s.	1st s.	2nd s.	1st s.	2nd s.	1st s.	2nd s.	1st s.	2nd s.	1st s.	2nd s.
Control without water		36.88	38.66	4.66	4.66	33.66	33.00	49.33	45.85	12.24	15.97	2.89	2.49
Control with water		41.33	42.00	5.00	5.66	35.00	32.66	53.95	52.80	22.80	25.49	2.42	2.47
Gamma rays	20Gy	46.66	57.33	8.33	8.00	47.66	47.00	64.93	63.67	35.15	36.11	4.81	4.70
	40 Gy	55.66	54.66	7.88	8.00	46.33	46.00	52.32	58.79	25.41	28.79	3.81	3.94
	80Gy	35.00	38.33	4.66	5.00	28.33	30.00	42.92	45.04	19.44	21.05	1.57	1.65
	Mean	45.48	50.11	6.77	7.77	41.67	42.33	53.39	48.61	25.34	26.32	3.06	3.43
Colchicine	0.1%	54.00	58.33	11.00	12.00	52.00	53.00	87.76	88.80	34.69	35.44	7.11	8.31
	0.2%	54.00	56.66	13.33	13.33	47.00	50.66	103.2	98.25	35.52	37.12	7.83	8.68
	Mean	49.78	52.33	9.77	10.33	45.15	45.78	81.65	79.96	31.00	32.69	5.45	6.49
Microwave	10 sec	65.00	68.33	9.33	11.33	32.66	44.00	100.9	96.93	28.21	29.03	7.16	6.05
	20 sec	55.00	53.3	9.00	8.66	31.00	33.67	94.51	95.38	26.78	24.87	4.38	4.49
	30 sec	36.33	38.00	4.00	4.66	27.33	30.00	71.26	68.37	17.60	17.32	2.79	3.24
	Mean	52.11	53.22	7.44	8.22	33.07	35.88	92.59	89.9	24.20	23.74	4.78	4.64
L.S.D. at	5%	2.94	6.83	1.80	1.82	2.94	7.14	4.23	9.02	1.32	4.65	0.90	0.91

3. **The flowering growth:**

The mean values of the flowering characters are shown in Table 3. It was observed that the flowering was found earlier with gamma ray at 20 Gy and microwave at 10 second as compared to control. but, colchicine at 0.1 and 0.2%, it was significantly decreased Number of days to first Flowering opening it took maximum days to flowering (109.66 and 109.0 days). comparing with control gave (105.66 and 105.0 days) in the two seasons**.**

On other hand, Flowering characters was affected by all tested treatments were highly significantly increased Flower spike fresh weight, Florets number per spike, Flowering zone length, Flower spike length comparing with the control treatment in both seasons. With respect to vase life response to all treatment on flower vase life, The maximum increase was achieved with colchicine at 0.2%, microwave at 10 second comparing with control in the two seasons. whereas, gamma ray at 80gray was significantly decreased in this respect in the first and second seasons **.** Other tested treatments showed an intermediate values in this concern in both seasons. The differences between all treatment were statistically significant in both seasons. While, treated corms of Gladiolus by gamma ray at 20 Gy due to gamma rays was may be due to the reduction in rate of physiological processes which assists in synthesis of flower inducing substances. Present findings are in agreement irradiated corms of gladiolus and observed that corms treated with lower doses resulted in early flowering. Present results are also in line with **Cantor et al., (2002)** who observed that gamma doses increased root and shoot length, which probably absorb more nutrient and produced more photosynthesis and ultimately resulted in early spike emergence and flowering. **Rather and John (2000)** also studied days to floret emergence in Dutch iris. Some doses of gamma rays resulted in early floret emergence; however, difference was not significant to the control. Similar observation has also been made by **Misra *et al.*, (2009)** who find out early bud initiation in chrysanthemum when various gamma doses applied than control. The present findings are also in line on days to flowering in gladiolus cvs. Sylvia and Eurovision. recorded early flowering with 20 Gy and 40 Gy in comparison to control on days to flowering. Present results are also in line with **Cantor *et al.*, (2002)** who observed that gamma doses increased root and shoot length, which probably absorb more nutrient and produced more photosynthesis and ultimately resulted in early spike emergence and flowering. also studied days to floret emergence in Dutch iris. Some doses of gamma rays resulted in early floret emergence; however, difference was not significant to the control. Delay in flowering due to gamma rays was may be due to the reduction in rate of physiological processes which assists in synthesis of flower inducing substances. **(Srivastava et al., 2007)**, who recorded early flowering on Gladiolus with 20 Gy and 40 Gy in comparison to control on days to flowering. **Sudha, and Dhaduk (2009)** stated that early flowering at lower doses of gamma rays may be because of physiological changes which occur in plant and delayed flowering occur at higher doses due to inhibitory effect. The present findings are also in line on days to flowering in gladiolus cvs. Sylvia and Eurovision **Kuldeep *et al.*, (2017).**

Table, (5) Effect of Gamma rays, Colchicine and Microwave on Flowering of *Gladiolus grandiflorus* plant during two seasons 2015/2016 and 2016/2017.

Character		No. of days to first Flow opening		a Spike length in(cm)		Flowering portion length (cm)		Florets number/spike		Florets fresh weight (gm)		Florets dry weight (gm)		Vase life	
seasons / Treatment		1st s.	2nd s.	1st s.	2nd s.	1st s.	2nd s.	1st s.	2nd s.	1st s.	2nd s.	1st s.	2nd s.	1st s.	2nd s.
Control without water		105.66	105.0	29.00	30.00	19.00	15.00	10.66	11.00	17.74	17.85	6.19	5.99	11.33	12.33
Control with water		100.00	104.3	39.66	41.33	24.66	27.67	13.33	11.33	18.92	21.87	5.77	5.73	9.00	8.44
Gamma rays	20Gy	75.66	73.66	56.66	59.66	30.66	33.66	17.66	18.00	30.30	32.21	7.86	8.64	16.00	16.00
	40 Gy	76.00	77.83	46.33	47.33	28.00	26.00	12.00	13.66	23.53	25.38	6.55	6.88	13.88	14.00
	80Gy	82.83	79.35	37.33	36.33	17.00	17.33	8.00	8.66	18.72	19.00	5.74	5.67	8.33	8.00
	Mean	78.75	74.15	46.78	47.78	25.21	25.72	12.60	13.00	24.18	25.53	6.72	7.07	13.40	13.66
Colchicine	0.1%	109.66	109.0	72.66	75.33	42.00	44.33	30.00	34.00	36.81	39.01	10.99	11.00	16.33	16.00
	0.2%	106.33	106.6	78.00	81.33	44.33	43.33	33.00	35.66	41.90	44.58	12.50	12.60	19.33	19.00
	Mean	103.7	105	64.11	66.00	37.00	38.40	25.40	27.00	32.54	35.15	9.76	9.78	14.88	14.48
Microwave	10 second	91.66	88.33	69.83	76.33	36.00	37.33	32.00	33.33	51.80	54.23	11.94	12.67	18.00	18.00
	20 second	96.66	96.33	60.33	61.66	30.00	29.33	27.00	28.66	47.00	47.58	7.33	9.65	16.33	15.00
	30 second	107.66	107.0	40.66	37.66	22.33	21.00	15.00	15.33	20.56	21.84	4.95	5.46	10.33	9.66
	Mean	98.66	97.22	58.11	58.56	29.41	29.20	24.71	26.00	39.78 89	41.22	8.08	9.26	14.88	14.22
L.S.D	at 5%	4.072	4.492	2.517	4.492	2.647	4.795	2.362	2.451	2.728	5.170	1.019	1.989	2.672	3.029

1. Corms and cormels production characters:

Data presented in Table (2) exhibit that colchicine at 0.1% and 0.2% for 20 hours. resulted the widest diameter (cm), heaviest fresh weight (g), new corms, as well as the highest number of Corm and cormels/plant comparing with control and other treatments in the two seasons, except for gamma rays at 40 and 80 Gy and Microwave at 30 second. Treatment colchicine at 0.1% concentration which gave the best number of corm and cormels in the first and second season closely near to that of the previous best treatment. microwave at 10 second produced the highest number of corms / plant followed by colchicine at 0.1% and 0.2% treatment gave the next results. whereas, Gamma rays at 20 Gy produced the third value in this concern, in both season. These results of nutrients agree with those obtained by **EL-Esawy *et al*., (1995)** on gladiolus**, Patil (2009)** on gladiolus**, and Srivastava *et al.*, (2007)** on gladiolus obtained that an increase in number of corms of gladiolus plants as a result of inoculation with a symbiotic increased by low gamma doses (5-25gray). Low level of mutagen itself is not responsible for early sprouting of gladiolus but it influences the activity of enzymes. Enzymes play an important role in plant metabolism to accelerate metabolism activities and consequently result in stimulating plant growth**,** This early sprouting of gladiolus corms at lower dose of gamma rays was probably related with the increase in the activities of gibberellins and auxins and disappearance of inhibitors**. Karki and Srivastava (2010).** Also, **Banerji and Datta (2002)** in gladiolus cultivar ‘White Friendship’ when the corms are exposed to gamma rays treatment. Enlargement and improvement in size of corm due to application of gamma irradiation was noticed by **Jun, and Fang (2007)** in Saffron. Reduction in corm size , At higher doses of gamma rays more production and bigger size of corms were obtained while higher doses resulted in smaller sized corms than control in Tuberose **Ali, (2002).**

Table, (6) Effect of Gamma rays, Colchicine and Microwave on corm and cormels production of *Gladiolus grandiflorus* plant during two seasons 2015/2016 and 2016/2017.

Character		Corms number/p		Corms fresh weight(g/corm)		cormels weight(g)/p		Corm diameter(cm)		Cormels number/p	
Treatment \ Season		1st s	2nd s	1st s	2nd s	1st s	2nd s	1st s	2nd s	1st s	2nd s
Control		1.00	1.00	18.65	20.63	4.01	3.96	3.53	3.50	14.66	15.67
Gamma	20Gy	1.66	1.66	38.86	41.13	9.49	10.54	5.33	5.70	38.33	41.00
	40Gy	1.00	1.00	29.96	33.56	8.40	8.61	4.73	4.66	32.00	35.33
	80Gy	1.00	1.00	23.18	23.83	4.39	4.29	4.06	4.20	25.00	23.33
	Mean	1.22	1.22	30.66	32.84	7.43	7.81	4.71	4.85	31.8	33.2
Colchicine	0.1%	1.66	1.66	47.78	57.58	11.57	11.81	4.50	5.13	33.33	39.33
	0.2%	1.66	1.66	49.71	57.80	10.44	11.15	5.48	5.83	37.66	44.66
	Mean	1.44	1.44	38.63	45.12	8.85	9.11	4.40	4.78	28.6	33.2
Microwave	10 sec	1.67	1.67	44.84	50.49	9.67	10.46	5.96	6.40	36.00	45.66
	20 sec	1.00	1.00	38.75	45.01	6.73	7.55	4.80	4.90	31.00	33.00
	30 sec	1.00	1.00	14.61	16.4	1.95	2.51	3.30	3.40	17.66	16.66
	Mean	1.22	1.22	32.73	37.29	6.12	6.84	4.68	4.90	28.2	31.8
L.S.D. at.05		0.63	0.67	2.677	3.291	1.059	0.885	0.357	0.361	2.999	3.713

Cytological studies:-

The presence of chromosome aberrations in root tip cells from *Gladiolus grandifloras* plant, both control and irradiated treatments were investigated. After radiation, a series of chromosome aberrations appeared, e.g., in most cells polyploidy, aneuoploidy, few cells contain micronuclei, chromosome bridges, chromosome fragments and lagging chromosomes (Figure 1). A quantitative difference in the number of abnormal cells was observed as a function of radiation dose. With an increase in the radiation dose, the percentage of total abnormal cells with chromosome aberrations were as chromosome breakage, lagging chromosome and fragment chromosome. The highest percentage of total abnormal cells appeared at 40 Gy. In these abnormal cells, irregular distribution of chromosomes in the two poles were the most frequent aberration type, and chromosome fragments were the second most important aberration type. The number of abnormal cells with micronuclei, chromosome bridges and chromosome fragments increased with increasing radiation dose. However, the number of abnormal cells with lagging chromosomes increased with increasing radiation dose only up to 40 Gy. A significant difference in the number of abnormal cells was observed as a function of colchicine treatment. With an increase in the colchicine treatment, the percentage of total abnormal cells containing polyploidy was significantly increased Figure (2).

1- **Physical mutagens:**

Fig.(1) Effect of Gamma rays on root tip cell of *Gladiolus grandiflorus*

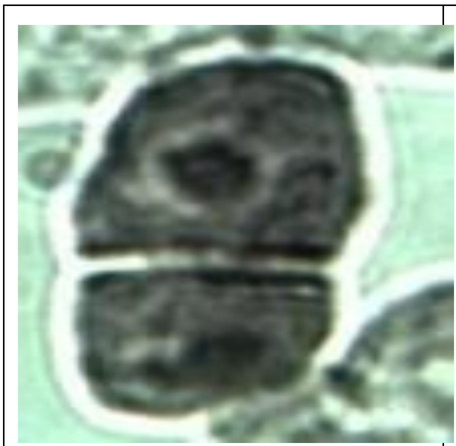
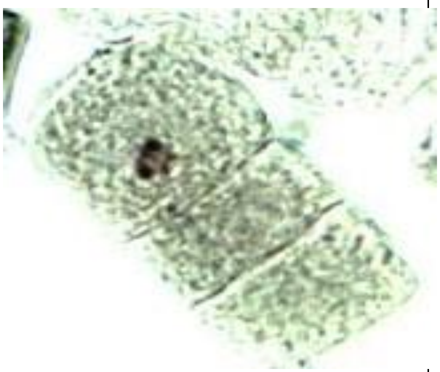
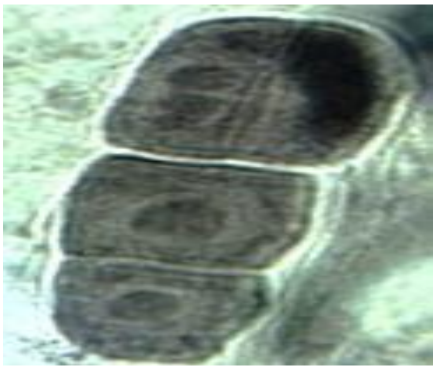
		
A Sticky chromosomes in interphase	B Side view of normal metaphase	C Sticky nucleus in interphase
		
D Sticky chromosomes in interphase	E More than one nucleus in interphase	F Interphase and metaphase

Photo (2): showing the abnormalities in Gladiolus after treatment with different doses of gamma rays. (a) sticky chromosomes in interphase (b): side view of normal metaphase. (c) sticky nucleus in interphase (d) sticky chromosomes in interphase. (E) more than one nucleus in interphase. (F) interphase and metaphase.

Fig. (2) Effect of Electromagnetic waves (Microwave) on root tip cell of *Gladiolus grandiflorus*

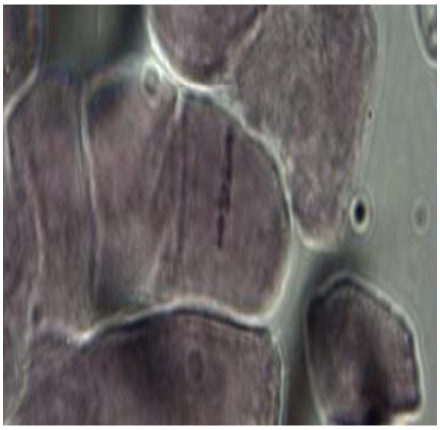
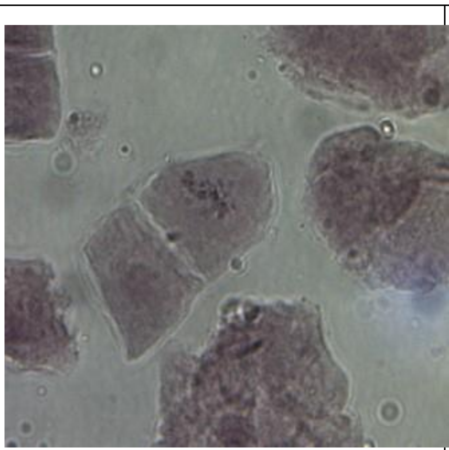
		
A Normal metaphase	B Polar view of metaphase with lagging chromosomes	C Lagging chromosomes in metaphase
		
D Lagging chromosomes in metaphase	E Normal metaphase in duplicated chromosomes	G Beginning of Anaphase

Photo (3): showing the abnormalities in Gladiolus after treatment with Microwave. (a)side view of normal metaphase, (b): polar view of metaphase with lagging chromosomes. (c) lagging chromosomes in metaphase and (d) unequal distribution of chromosomes with lagging chromosomes in metaphase (E) normal metaphase with duplicated chromosomes and (G) Beginning of chromosomes in Anaphase separation.

1- **Chemical mutagens:**

Fig. (3) Effect of Colchicine on root tip cell of *Gladiolus grandiflorus*

		
A Tetraploid number (n =60)	**B Hexaploid number (n=90)**	**C lagging ring chromosomes (n=30)**

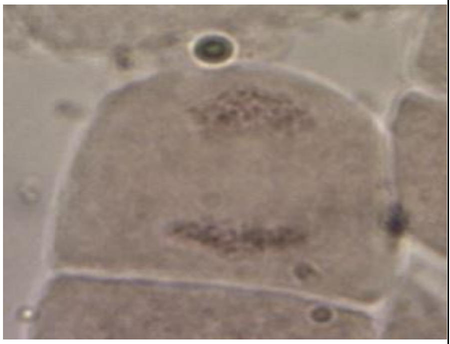
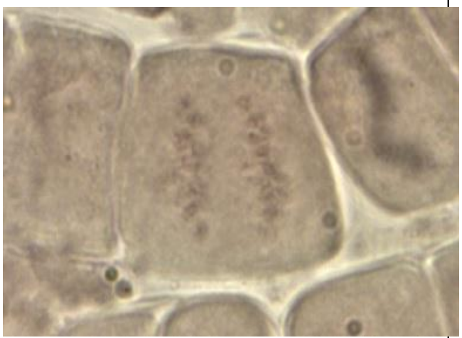
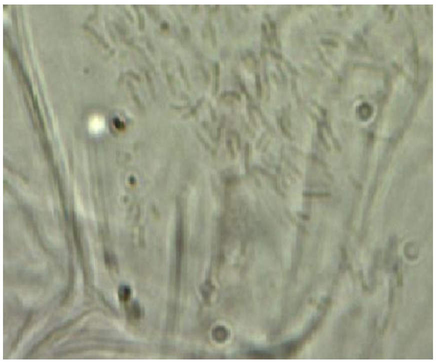
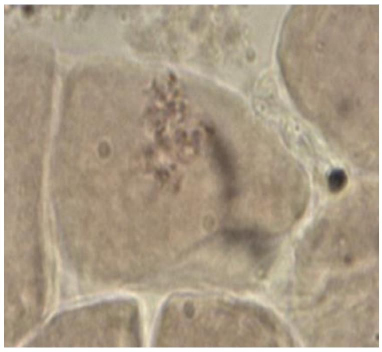
		
E Duplicated number of chromosomes	**D Tetraploid number (n=60)**	F Duplicated number of chromosomes
		
G Anaphase with unequal distribution of chromosomes	H Normal side view metaphase	**I** Normal side view metaphase
		
J Normal side view metaphase	K many duplicated chromosomes	Y polar view of metaphase

Photo (1): showing the abnormalities in Gladiolus after treatment with different concentrations of colchicine. (a) and (b): side view of metaphase. (c) and (d) unequal distribution of chromosomes during telophase with sticky. (E) Duplicated number of chromosomes (F) Duplicated number of chromosomes (G) and (I): side view of metaphase. (J) and (K) many duplicated chromosomes (Y) polar view of metaphase during telophase with sticky.

Fig. (4)Effect of Gamma rays and colchicine on *Gladiolus grandiflorus*

		
flower spike Length compared with control	**flower zone Length compared with control**	**Large sized floret**
		
Flower color and flowering attributes (orange petal)	Effect of colchicine at 0.2% on Gladiolus. (large florets)	80 Gy abnormal growth of spikes

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