

## EFFECT OF GAMMA IRRADIATION, METHYL METHANE SULPHONATE AND THEIR COMBINATIONS ON GROWTH, FLOWERING AND INDUCED VARIABILITY IN *Tagetes erecta*, L.

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### ABSTRACT

Seeds of *Tagetes erecta*. were irradiated with different doses of gamma-rays (0.5, 10 and 15 kr.), other part of seeds was treated with different levels of MMS (0.0, 0.1, 0.2, 0.3, 0.4, 0.5 and 1.0%). Also there were combined treatments between the two mutagens..

Intermediate doses of gamma-rays (10kr.) gave the lowest germination percentages in both seasons. There were no significant differences among the different treatments of gamma-rays in M<sub>1</sub>- and M<sub>2</sub>-generations of both seasons- on the plant height. All doses of gamma-rays delayed the flowering date in all generations for both seasons. Except the M<sub>2</sub>-generation of the first season, gamma-rays showed significant effect on the number of flowers in all generations. 5 kr. in the M<sub>1</sub>-generations of both seasons had the largest average number of inflorescences, but in the M<sub>2</sub>-generation of the second season, it had the lowest one. Low doses of gamma-rays (5 kr.) caused smaller flowers than normal in the M<sub>2</sub>-generation of the first season.

Low concentrations of MMS (0.1%) caused a stimulating effect on seed germination in both generations. But the high concentration (1.0%) caused death of seeds (lethal dose). Different treatments of MMS showed no significant differences in M<sub>1</sub>-generation in both seasons on the plant height. High concentration of MMS (0.5%) caused albino chlorophyll mutant in the seedlings in the M<sub>2</sub>-generation of the first season. Low concentration of MMS (0.2%) delayed the flowering date in all generations with one exception in the M<sub>2</sub>-generation of the second season, where 0.5% was the latest one. Low concentration of MMS (0.1%) gave the largest flower number in the M<sub>1</sub>-generation for both seasons but the high concentration (0.5%) gave the largest one in the M<sub>2</sub>-generation of the first season.

The combined treatments were more efficient than the single ones on seed germination. The lethal dose ranged between the doses 1.0% MMS + 0. Kr. gamma-rays to 1.0% MMS + 15 kr. gamma-rays. Generally, 0.5% MMS + 5 kr. gamma-rays gave the largest number of inflorescences in all generations except in the M<sub>2</sub>-generation of the second season. Many different petals shape were obtained from the different combined treatments. Seeds of M<sub>1</sub>-plants treated with 10 kr. + 0.3% resulted in a mutation in stalk shape.

### INTRODUCTION

*Tagetes erecta* is a member of the family *Compositae*. , it is considered as a strongly aromatic, half-hardy annual of branching habit with pinnate, pungent, deeply cut leaves and large single, yellow or orange daisy-like

flowers up to 10 cm across on 60 cm stems. The flowering season is from summer to early autumn or even later in tropical and subtropical climates. *Tagetes erecta* can be used for bedding , annual borders, containers etc., seeds can be sown outside where they are to flower under full sun. Being a sturdy plant, it adapts itself to most types of soil. To get novel genetic variability which is necessary for further plant improvement in this plant, induced mutations should be tried either by using physical or chemical mutagens.

The main objective of the present investigation was to study the effects of different doses of acute gamma-radiation, from cobalt-60 , different levels of MMS and combination between them on some vegetative growth and flowering characters of *Tagetes erecta* in order to obtain variation in *Tagetes erecta* which can be of value for improving this plant.

## **MATERIALS AND METHODS**

The experiments were carried out during 1995, 1996 and 1997 in the Floriculture and Ornamental Horticulture Research Branch , Antoniades Garden, Alexandria , Egypt. Two series of experiments were conducted . The first dealt with the M1-generation while the second dealt with the M2-generation.

### **M1-generation**

Seeds of *Tagetes erecta* , L. “a local cultivar” selfed for 7 generations were used in these experiments. These seeds were obtained from the Floriculture and Ornamental Horticulture Division , Horticulture Research Institute, Cairo ,Egypt. Gamma-rays used in this study were generated from the cobalt-60 source, in Gamma-Cell installed in Irradiation Laboratory at Middle East Regional Radio-isotope Center for the Arab Countries at El-Dokky , Cairo, Egypt. Methyl-methane-sulfonate (MMS) [CH<sub>3</sub>SO<sub>4</sub>CH<sub>3</sub>] used in this study was obtained from Merck , Germany. M=110.13 g/mol.

The layout of the experiments was designed to provide complete randomized blocks in factorial experiment containing three replicates (Snedecor and Cochran , 1967) . Each replicate contained 28 different treatments for M1-generation during 1995 and 1996. One hundred seeds was used for each treatment in every replicate (28x100x3=8400 seeds ).

The dry seeds were exposed to different doses of gamma-rays from Co-60 in Gamma-Cell on March 6, 1995 and March 17, 1996 in the first and second seasons respectively. The used doses were 0,5,10 and 15 Kr. Seeds were treated with methyl-methane sulfonate (MMS) solution on March 19, 1995 and March 20, 1996 for the first and second seasons respectively. These seeds were soaked in 0.0, 0.1, 0.2, 0.3, 0.4, 0.5 and 1.0 % MMS solutions in Petri dishes containing 15 cm<sup>3</sup> of solution for 6 hours. The treated seeds were washed with tap water and sown on the same day directly in seed-pans containing 1:1 peat moss and sand and set in a greenhouse and watered throughly.

The germination ratios were recorded in the laboratory, using Petri dishes containing wet filter papers. Thirty seeds were sown in each dish on March 19, 1995 and March 20, 1996 in the first and second seasons respectively. Three replicates were used and each replicate contained 28 treatments.

After five weeks, the seedlings were transplanted into 15 cm. diameter plastic bags containing the same soil mixture. These bags were set in the plastic house and watered thoroughly. Four weeks later, the plants in the plastic bags were finally transplanted to the open field which was fertilized with organic manure and divided into three equal parts, each part was used as a replicate. Every replicate contained 28 treatments. Three plants were used as an experimental unit for each treatment within every replicate. The M1-field experiments were terminated on October 29, 1995 and October 25, 1996 in the first and second seasons respectively.

Unless otherwise stated, the following parameters were recorded for the M1-generation in the two successive experimental seasons:

Seeds germination percentage, plant height (cm), chlorophyll content of the leaves (mg/100g), fresh weight of leaves according to Arnon, 1949 and Wellburn, 1994), flowering date (the number of days between sowing and the appearance of the first inflorescence on the plant), number of inflorescence, and inflorescence diameter (cm).

**Variation in the M1-generation** : Changes in the vegetative growth or inflorescence at each treatment were recorded. These changes included habit of growth and inflorescence form.

The mean comparisons were according to Duncan's multiple range test at the 5% level of probability. For germination percentage, angular transformation was settled and the statistical analysis was carried out using values resulting from transformation, then the original values were marked with the alphabetical letters of significance. Heterogeneity between the variance of each mutagenic treatment and the control was tested using two-tail F-test at the 5% level of probability.

### **M2-generation**

About one third of the flowers produced on M1-plants in each treatment were selected and selfed. All visible changes, off-types and abnormal M1-plants were not selected. The produced seeds of all selected and selfed flowers in M1-plants from each mutagenic treatment were collected. The collected seeds from M1-generation were sown in seed-pans on March 23, 1996 and March 22, 1997 for the first and second seasons respectively. A sample of 210 seeds from each treatment was sown in 3 seed-pans (70 seeds/ seed-pan). The seed-pans were set in the greenhouse and watered thoroughly. After five weeks, seedlings were transplanted to a black plastic bags containing the same soil mixture used before (1:1 peat:sand by volume). After four weeks, the plants were finally transplanted to the prepared field. 6 and 4 replicates were used in the first and second seasons respectively. Each replicate contained 27 and 24 treatments for the first and second seasons respectively. Three plants were used as

experimental unit. All characters of M2-generation were measured in the same manners mentioned in the M1-generations.

**Variation in the M2-generation :** These changes included: Habit of growth, Inflorescence colour, Inflorescence form, and Peduncle shape.

## **RESULTS AND DISCUSSION**

### **Seed Germination**

Generally, there was an apparent trend toward decreasing the germination percentage with increasing the doses of gamma rays more than 5Kr. which significantly increased the percentage of *Tagetes erecta* seed germination as compared with the control and other treatments in both generations of the two seasons, with one exception in the M1 of the first season( Table 1). The previous results may be due to the activation of enzymes or the encouragement of the embryo cell division or both of them. These results are in agreement with those obtained by Zaharia *et al.* (1991) on *Tagetes erecta* .

The percentage of germinated seeds significantly decreased with increasing the doses of MMS as compared with the control with one exception of 0.1% MMS treatment which was higher than the control in all generations except for M1-generation in the first season. These results are in agreement with those obtained by Misiha and Hussein (1992).

The stimulating effect of the low concentrations of the used chemical (MMS) on the seed germination may be due to a physiological influence. The first phase of germination is the swelling of cells by hydrates followed by enzymatic activation and metabolism. The material and energy necessary for this initial growth are already available in the seed, and so the young embryo has no need to form new substances, but only to activate through those already stored in the cotyledons. The role of the chemical may be increasing of enzymatic activation and awakening meristemic cell division in the seed.

The reduction in the percentage of germinated seeds as a result of treating with high concentrations of chemicals are related to the damage of embryo which might arise from ionizing chemicals. Also this reduction may be due to the effect of the high concentrations which inhibits the synthesis of enzymes. This damage or inhibition result in a decrease in the germination rate.

The effects of the combined treatments were highly significant in all generations except in the M1-generation of the first season. The lethal dose ranged between the doses 1.0%MMS+0Kr. to 1.0%MMS+15Kr. These results are similar to the findings of Dixit and Dubey (1988) on *Lens culinaris*, Boncheol and Maluszynski (1997) on barley and Kumari and Singh (1997) on *Pisum sativum*.



### **Plant height**

The results presented in Table 2 show that there were no significant differences among the different gamma radiation treatments in the M1 and M2-generations of both seasons. These results seemed to agree with those reported by Kamal *et. al.* (1975) on *Strelitzia reginae* and Omar (1995) on *Gomphrena globosa*. Many workers reported that the plant height was stimulated as a result of the effect of low and intermediate gamma-ray doses, for example Badr *et.al* (1978) on *Lycopersicon esculentum*, Mill., and Hegazy (1980) on *Phaseolus vulgaris*, L., .On the other hand, other workers noted that gamma-rays inhibited the height of different plants such as, *Cajanus cajan*, Mill sp. (Chowdhury and Sharma, 1985) , *Vigna aconitifolia* (Kothekar, 1991) and *Plantago ovata* at high doses (Sareen and Koul , 1994).

Regarding the M1-generation in both seasons, the different treatments of (MMS) showed no significant effects. In the M2-generations in the two seasons, there was a stimulating effect at 0.2% and 0.3% compared with the control. On the contrary, the other doses decreased plant height.

The stimulated effect of the lower concentrations on the plant height agrees completely with the findings of Floria *et.al* (1984) on *Datura innoxia* Mill, Abdel-Maksoud and El-Mahrouk (1992) on *Asparagus densiflorus* and Al-Halawany (1992) on *Catharanthus roseus* (L.). The proportional decrease in the plant height with increasing the MMS concentrations found in the present study was supported by many investigators on other plants ,i.e. Sallam *et al.* (1975) on *Strelitzia reginae* and El-Nashar (1998) on two cultivars of *Tagetes erecta*. These results might be attributed to the physiological damage caused by MMS and its hydrolysis products.

The effects of the combined treatments were highly significant in M1- and M2- generations of the first season only. In this study, the data indicated that most of combined treatments caused significant negative shifts in the mean values of plant height with some exceptions at 0.2%+10Kr. in the M1- and M2-generation respectively which gave plants taller than the control . These findings are in harmony with those obtained by Dixit and Dubey (1988) on *Lens culinaris* , Misiha and Hussein (1992) on *Althaea rosea* and Gataulina (1996) on *Lupinus albus*. On the contrary, the stimulating effect was similar to that found by Dixit and Dubey (1988) on *Lens culinaris*. These results may be due to the balance between the stimulating effect of the lower doses of mutagens, and the inhibiting effects of their higher doses.

### **Leaves chlorophyll content**

The frequency and spectrum of chlorophyll mutations recovered in the M2-generations were taken as an indicator for the effectiveness and efficiency of the mutagenic treatments employed ( Table 3). It is clear that MMS is relatively more effective and efficient than gamma-rays in inducing higher frequency of chlorophyll mutations. Similar findings were reported by Hussein *et al.* (1974) , Misiha (1982) , and Misiha and Hussein (1992). It is known that the changes in chlorophyll content is associated with the changes in the chloroplasts (Evans, 1984). The important factors that control chloroplast differentiation area are:





Genetic information present in plastids which contain the chloroplast DNA (Kirk and Tilney-Basset, 1978), (2) Cytokinins have been shown to control chloroplast differentiation independently of their action on cell division (Seyer *et al.*, 1975) and (3) inorganic salts (iron, magnesium, copper, potassium and ammonium salts) play important roles in the synthesis or metabolism of chlorophyll in plants (Steward, 1963).

The effect of MMS which resulted in chlorophyll mutant can be attributed to enhancement in chloroplast differentiation or any other reason from the previous ones.

#### **Flowering date**

Data reported in Table 4 show that the flowering date was affected by the different doses of gamma-rays. There were highly significant differences among the treatments in all generations for the two seasons. All mutagen treatments resulted in a delayed flowering date as compared with the control. These results were in conformity with those reported by Kothekar (1991) on *Vigna acontifolia* and Omar (1995) on *Gomphrena globosa*. The mechanism of floral initiation is a dramatic event involving a total change over the character and developmental pattern of the meristem. There are many discussions about florigens, or flower induction substances, that act in the doses which enhanced the beginning of flowering. In this study, it may be explained as a result of delaying or inhibiting the synthesis of florigens which resulted in an increase by the number of days to flowering under the external environmental conditions because the induction of flowering can be affected by many factors and varied and may be either internal or external (Bidwell, 1979).

Plants treated with 0.2% MMS were the latest ones in flowering in all generations with one exception in the M2-generation of the second season, where 0.5% was the latest one. These results are similar to the findings of Hussein *et al.* (1974) on *Salvia splendens*; Gupta *et al.* (1982) on *Pennisetum americanum*, Khan *et al.* (1995) on mungbean and El-Nashar (1998) on *Tagetes erecta*. Generally, high concentrations of chemicals seemed to inhibit cell growth, decrease the rate of growth and delay the flowering as found and reported by Vandana and Dubey (1993) and Khan *et al.* (1995).

The obtained data showed that there were significant differences among the combined treatments of gamma-rays and MMS in all generations. The results showed that the time to flowering was increased by increasing the doses. The combined treatments was more effective in inhibiting the flowering than the single treatments. Similar results were reported by Kamel *et al.* (1984) on *Hunnemania fumariaefolia* and Misiha and Hussein (1992) on *Althaea rosea*.

#### **Inflorescence diameter**

Gamma-rays significantly affected the flower diameter in the M2-generation of both seasons, but there were no significant differences in the M1-generation of both seasons (Table 5). There was an apparent trend towards decreasing the flower diameter with increasing the doses of gamma-rays. The previous results may be attributed to a reduction in the cell number and/





or size. These results support the findings of Lata (1980) on *Rosa spp.* Banerji and Datta (1987) on *Hibiscus rosa-sinensis*,L. and Omar (1995) on *Gomphreana globosa*.

On the other hand, MMS did not significantly affect the flower diameter in all generations with one exception in the M1-generation of the second season. These results were in agreement with those reported by Al-Halawany (1992) on *Catharanthus roseus* and El-Nashar (1998) on *Tagates erecta*. In the M1-generation of the second season, MMS concentrations increased flower diameter compared with the control. These results may be as an effect of environmental and physiological conditions at the middle of the flowering period which can influence the flower quality. The vegetative growth reaches its maximum, the photoperiod was longer than any other time and the light intensity and temperature were higher. Therefore, the photosynthesis and the production of carbohydrates and energy were at the ideal rate, which led to the improvement of the flower quality and increase its diameter and give the chance to the chemical agents to affect this characteristic.

. The data revealed that the combined treatments were effective than the single ones and also that gamma-rays was the main source in inducing variations in flower diameter. Some differences may be due to the environmental and physiological conditions. These results are similar to that reported by Misiha (1982) on *Phlox drummondii*.

#### **Number of inflorescences**

The dose of 5Kr. produced the largest average number of inflorescences in the M1-generation of both seasons, which significantly differed from the other treatments including the control (Table 6). On the contrary, in the M2-generation of the second season, the same treatment had the lowest average number of inflorescences. Such different effects of the mutagen were also reported by Stepanenko and Regir (1983) on *Calendula officinalis*,L., Lal *et al.* (1993) and Omar (1995) on *Gomphrena globosa*. Bidwell (1979) mentioned that all steps in the flowering process are preprogrammed in the totipotent cells of the meristem. All that is needed as trigger or a release that sets these cells on the way in the program for flowering. The capacity to flower is inherent, like the capacity to form leaves.

The treatment 0.1% MMS gave the largest flower number in the M1-generation of both seasons but 0.5% had the largest one in the M2-generation of the first season. These results are in conformity with Al-Saheal and Gamil (1982) on bread wheat; and El-Nashar (1998) on *Tagates erecta*. The differences between the results of the two generations might be due to climatic factors.

The results obtained from the combined treatments indicated that there were significant effects among the different treatments. Generally, 0.5% MMS + 5Kr. gamma-rays gave the largest number of inflorescences in all generations except in the M2-generation of the second season. These results may be due to the stimulation effect of 5Kr. alone or 0.5% alone which was mentioned before and/or due to the stimulating effect confirmed by the combining.



### **Inflorescence colour**

In the M2-generation of the first season, the results showed that there were slight change in the flower colour by the treatments of 5Kr. and 10Kr. + 1.0%. There are frequent reports that ionizing radiation can affect the flower colour i.e. Stepankko and Regir (1983) on *Callendula officinalis*, Venkatachalam and Jayabalan (1991) and Venkatachalam and Jayabalan (1994) on *Zinnia elegans*. Some other workers reported that EMS cause morphological mutation involved flower colour such Al-Halawany (1992) on *Catharanthus roseous*. This change in the flower colour can be attributed to the effect of the mutagen treatment together with temperature and light on the development of pigments.

### **Inflorescence form and size**

Smaller flowers than normal were obtained by the treatment of 5Kr. and 15Kr. + 0.4% in the M2-generation of the first season, 10Kr. +0.1% and 5Kr. + 0.4% in the M2-generation of the second season. This may be due to that the ionizing radiation caused some changes in the shape and form of the floral organs such as those reported by Badr and Etman (1977) on *Dianthus caryophyllus*, L., Smilansky et al. (1986) on *Rosa spp.*, and Khalaburdin (1993) on *Canna hybrida*. Also, petals number can be attributed to the effect of the chemical on flower bud during the time of its initiation. Chemical treatment can induce changes in the size of ray florets as reported by Neagu (1974) on *Helianthus annus* and Vandana and Dubey (1993) on *Vicia faba*. The small flowers may be due to the effect of the mutagens on the whole flower bud which inhibited its growth.

### **Petals shape**

Many petals shapes were obtained in the M2-generation. These changes in petals shape may be due to that mutagens caused some changes in the shape and form of the floral organs which was also found by Rani and Jayabalan (1992) on *Tagates patula*. In the cases of the combined treatments, the changes may be attributed to the effect of the mutagens on flower bud during the time of its initiation. This result is in agreement with that of Misiha and Hussein (1992) on *Altheae rosea*.

### **Stalk shape**

In the M2-generation of the second season, plants emerged from seeds of M1-plants treated with 10Kr. + 0.3% resulted in a mutation in stalk shape which revealed that the combined treatments was more effective than the single treatments in inducing mutants by the stalk shape. It may be due to the harmful effect of the ionizing radiation and/or the effect of chemical mutagen on the structure of the flower stalk.

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تأثير أشعه جاما و المطفر الكيماوي ميثيل ميثان سلفونات (MMS) والتفاعل المشترك بينهم علي بعض صفات النمو الخضري والأزهار لنبات القطيفة *Tagetes erecta*

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تم اخذ جزء من البذرة وتم تشعيها بأشعه جاما بالجرعات صفر, 5, 10, 15 كيلو راد. وتم نقع جزء آخر من البذرة في المطفر الكيماوي ميثيل ميثان سلفونات MMS لمدة 6 ساعات في التركيزات صفر, 0.1%, 0.2%, 0.3%, 0.4%, 5%, 1%. وقد تم عمل معاملات مشتركة بين المطفرين. ..ويمكن تلخيص النتائج كما يلي:-  
-وجد أن الجرعات المتوسطة من جاما (10 كيلو راد) أعطت أقل نسبة إنبات وذلك خلال المواسم المختلفة.  
-لم تؤثر المعاملات المختلفة من أشعه جاما معنويا علي ارتفاع النبات.  
-أخرت كل الجرعات المستخدمة من أشعه جاما تاريخ الإزهار.  
- لوحظ أنه بزيادة جرعات أشعه جاما قلت أقطار النورات وذلك في الجيل الطافر الثاني M<sub>2</sub> بينما لم توجد أي فروق معنوية في الجيل الطافر الأول M<sub>1</sub>.  
-وجد أن المعاملة بـ 5 كيلو راد أدت إلى أكبر عدد من النورات وذلك في M<sub>1</sub> للموسمين ولكن في M<sub>2</sub> للموسمين أدت إلى أقل عدد نورات.  
-انتجت الجرعات المنخفضة من جاما (5 كيلو راد) إقل حجم للأزهار وذلك في M<sub>2</sub> للموسم الأول.  
- أدى التركيز 0.1% من المطفر MMS إلى زيادة نسبة الإنبات في حين أن التركيز العالي 1% أدى إلى موت البذرة.  
-وجد في M<sub>2</sub> للموسمين ان استخدام التركيزات 0.2%, 0.3% أدى إلى زيادة ارتفاع النبات في حين أن باقي المعاملات أدت إلى انخفاض ارتفاع النبات.  
- تسبب التركيز 0.5% إلى طفرة كلوروفيل بيضاء وذلك في البادرات الناتجة في M<sub>2</sub> للموسم الأول.. ومن ذلك يتضح أن المطفر الكيماوي أكثر فاعلية في إحداث طفرة كلوروفيل من أشعه جاما.  
-التركيزات المنخفضة من المطفر الكيماوي (0.2%) أخرت ميعاد الإزهار وذلك في كل الأجيال فيما عدا M<sub>2</sub> في الموسم الثاني حيث تسبب التركيز 0.5% في التأخير.  
-التركيز المنخفض من المطفر الكيماوي (0.15) أعطى أكبر عدد من النورات في M<sub>1</sub> لكلا الموسمين ولكن التركيز العالي (50). زأدى إلى أكبر عدد من النورات في M<sub>2</sub> للموسم الأول فقط.  
- المعاملات المشتركة كانت أكثر فاعلية في نسبة الإنبات عن أشعه جاما وحدها.. ولقد تراوحت الجرعات الممبئة من 1% + صفر كيلو راد إلى 1% + 15 كيلو راد.  
- أدت معظم المعاملات المشتركة إلى انخفاض في متوسطات ارتفاع النبات فيما عدا بعض الاستثناءات وهي 0.2% + 10 كيلو راد, 0.2% + 5 كيلو راد وذلك في الأجيال M<sub>1</sub>, M<sub>2</sub> تعلى الوالي التي أعطت نباتات أطول من معاملة المقارنة.  
-المعاملات المشتركة كانت أكثر فاعلية في تأخير ميعاد الإزهار عن المعاملات الفردية.  
-وجد أن المعاملة 0.5% + 5 كيلو راد أدت إلى أكبر عدد نورات في كل الأجيال فيما عدا M<sub>2</sub> في الموسم الثاني.  
- أدت المعاملة 1% + 10 كيلو راد إلى تغيير طفيف في لون الأزهار.  
-تم الحصول علي ازهار صغيرة عن معاملة المقارنة وذلك بالمعاملات 0.4% + 15 كيلو راد في M<sub>2</sub> للموسم الأول, 0.1% + 10 كيلو راد , 0.4% + 5 كيلو راد في M<sub>2</sub> للموسم الثاني..  
-تم الحصول علي أشكال متعددة من البتلات وذلك باختلاف المعاملات المشتركة.  
-تم الحصول علي طفرة في شكل الحامل النوري في M<sub>2</sub> للموسم الثانوذلك بعد استخدام المعاملة 0.3% + 10 كيلو راد.