MATERIAL AND METHODS

The certified, healthy and dry seeds of Ammi majus L. were used for all the experiments. For each treatment 200 seeds (five replicates each) were treated with different concentrations of chemical mutagens and irradiated with different doses of gamma rays. The concentrations of DES, EMS, caffeine and thiourea were 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0% and those of 2,4-D were 10, 20, 40, 60, 80, 100, 120, 140 and 160 ppm. The seeds were irradiated with 5, 10, 20, 30, 40, 50, 60, 80, 100, 120, 140 and 160 kR in gamma chamber having CO\(^{60}\) source at National Botanical Research Institute, Lucknow. One set of seeds was soaked in distilled water as control.

SEED GERMINATION

Germination counts were recorded on 15, 20 and 30 days after sowing when seedlings had emerged above the soil surface. In case of delayed germination the counts were made on 40 and even 46 days after sowing. The percentage germination is based on the number of seeds germinated and is calculated by the following formula.

\[
\text{Germination percentage} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds sown}} \times 100
\]
SEEDLING SURVIVAL

Seedling survival was calculated 30th day after sowing and values expressed as percentage over control. The decreased survival values indicated lethality. Seedling survival was calculated by the following formula.

\[
\text{Seedling survival (\%)} = \frac{\text{No. of seedlings survival 30th day after sowing}}{\text{Total no. of seeds germinated}} \times 100
\]

GROWTH PERFORMANCE

The height of 30 randomly selected seedlings of control as well as of all the treatments were taken on 20 and 30 days after sowing and when the mature plants had attained maximum height. The height was measured from the soil surface upto the tip of the oldest leaf to the nearest centimeter. The rate of the growth of seedlings was based on the height on 20th and 30th day after sowing.

Height Injury :

Height injury of seedlings was calculated on 30th day after sowing and in mature plants it was calculated at the time of flowering by the following formula.
Height Injury = \( \frac{HC - HR}{HC} \times 100 \)

where \( HC \) = height of control seedlings or mature plants.

\( HR \) = height of treated seedlings or mature plants.

**MORPHOLOGICAL ABNORMALITIES**

The morphological mutations were selected on the basis of visible physical characters and deviations from the normal behaviour of the plants as compared to control in \( M_1 \) and \( M_2 \) generations. The parameters of morphological mutations were size and shape of cotyledonary and vegetative leaves at seedlings stage, habit, branching, flowering and height of mature plants. Seedling abnormality is calculated as under.

\[
\text{Seedling abnormality (\%)} = \frac{\text{No. of abnormal seedlings}}{\text{No. of seeds germinated}} \times 100
\]

The scoring in \( M_1 \) material was confined mostly to seedling stage, because at this stage the morphological variations were easily scored.

**MUTATION FREQUENCY**

Following method was adapted to calculated mutation frequency.

\[
\text{Mutation Frequency (\%)} = \frac{\text{No. of mutated plants}}{\text{Total No. of } M_2 \text{ plants}} \times 100
\]
MEIOTIC ABNORMALITIES

Meiotic abnormalities were scored on the basis of structure and behaviour of cells and chromosomes. Any deviation from normal cell was considered as an abnormality like chiasmata frequency, univalents, multivalents, precocious separation, stickiness, laggards, bridges and fragments etc.

POLLEN FERTILITY

Pollen grains from mature anthers were stained in 1.0% acetocarmine. Stained and turgid pollen grains were counted as fertile. The shrunken, mis-shapen, empty, minute and unstained pollen grains were considered sterile. Decrease in fertility over control was put under induced pollen sterility. The mutagen sensitivities were compared with each other using a newly devised index, 'relative pollen sterility' (Yamagata et al., 1969), which was calculated by the following formula.

\[
\text{Relative pollen sterility} = \frac{r - c}{100 - c} \times 100
\]

where \( c \) = percentage pollen sterility in control.
\( r \) = percentage pollen sterility after treatment.

PLANT FERTILITY

Plant fertility was based on the output of healthy seeds per inflorescence. The average number of seeds produced by
control plants exhibited 100% plant fertility (maximum). Data from the treated plants were compared with control and expressed in percentage. Weight of per hundred seeds was also taken as a comparative account. Plant fertility was calculated by the following formula.

\[
\text{Plant fertility} (\%) = \frac{\text{output of seeds per treated plant}}{\text{output of seeds per control plant}} \times 100
\]

**PLANT STERILITY**

Induced sterility was calculated on the basis of the decrease in seed setting per inflorescence or per plant using the formula given by Konzak *et al.* (1968).

\[
\text{Plant sterility} (\%) = \frac{Sc - Sr}{Sc} \times 100
\]

where \( Sc \) = seeds per inflorescence in control.

\( Sr \) = seeds per inflorescence in treated plants.

**YIELD**

Yield was calculated from the total number of seeds per plant in control and treated plants. Increase or decrease in yield of treated plants over control was also calculated.
METHODS OF PREPARATION OF CHEMICAL MUTAGENS

Chemical mutagens were dissolved in distilled water according to the required concentrations. The solutions of diethyl sulfate, ethylmethane sulfonate, caffeine and thiourea were prepared in distilled water. 2,4-D was at first dissolved in minimum quantity of absolute alcohol and then diluted to required concentrations.

METHODS OF TREATMENTS

The seeds (200 seeds in 5 replicates) were soaked in each concentration of DES, EMS, caffeine, thiourea and 2,4-D for 24 hours. The solutions of the same concentrations were replaced after first 12 hours. The treated seeds were washed with water and sown in earthen pots having well manured soil. The pots were watered and weeded regularly. After 45–50 days the seedlings were transplanted in well prepared beds.

BUD FIXATION

Young flower buds were fixed between 8.00–11.30 a.m. in Carnoy's solution (6 parts absolute alcohol:3 parts chloroform:1 part acetic acid glacial) for 40–45 minutes. Buds were then transferred to propionic acid (saturated with ferric acetate) for 24 hours and then stored in 70% alcohol. Anthers were squashed in 0.5% propionocarmine, dehydrated in 1:1 NBA series (normal butyl alcohol:acetic acid glacial) and mounted in canada balsam.
Photomicrographs were taken from temporary as well as permanent slides.

EXPERIMENTAL DESIGN IN M₁ AND M₂ GENERATIONS

Randomised block design was adapted in M₁ and M₂ generations with five replicates. Each treatment and control in M₂ comprised of 50 randomly selected plants.

MACRO MUTATIONS

Morphological and other visible mutations in each treatment were scored as macromutations in M₁ and M₂ generations on the basis of:

1. Percent of M₁ plant progenies (segregating) and
2. Percent of M₂ mutated plants.

Much stress was given on morphological mutations.

MICRO MUTATIONS

The quantitative characters measured in M₁ and M₂ generations were:

1. Number of days required for germination after sowing.
2. Number of days for flowering.
3. Number of days for seed setting.
4. Number of days for seed setting and ripening.
5. Number of days required between (a) completion of germination and flowering (b) flowering and seed setting (c) seed setting and ripening (d) over all life span of the plants in different treatments.

STATISTICAL ANALYSIS

The observations recorded on different characters relating to each of the different treatments have been subjected to statistical analysis with a view to find the individual and comparative effect of different mutagens. In other words an effort has been made to find whether the expression of characters, as shown by its mean value estimated from the entire population of M₁ and constituted by the M₂ plants, has been affected by the different mutagenic treatments.

MEAN, STANDARD DEVIATION AND COEFFICIENT OF VARIATION

Mean: It is a measure of central tendency of distribution and defined as the sum of all individual observations divided by the number of observations.

Standard Deviations (SD, σ): It is positive square root of the average of sum of squares of deviations of all observations from their means. It is calculated by computer based on the formula.
\[ SD(\sigma) = \sqrt{\frac{\sum (x_i - \bar{X})^2}{n}} \]

or \[ SD(\sigma) = \sqrt{\frac{\sum (x_i - \bar{X})^2}{n}} \]

where \( x \) = individual reading.

\( \bar{X} \) = mean of all the readings.

\( n \) = number of replicates.

**Coefficient of Variations (CV)**: It measures the relative magnitude of variation present in observations relative to the magnitude of their arithmetic mean. It is defined as the rate of standard deviation to arithmetic mean expressed as a percentage.

\[ CV = \frac{SD}{Mean} \times 100 \]

It is concluded that smaller the CV, more consistent or less variable is the data and vice-versa.

**TEST OF SIGNIFICANCE**

It determines how far their mean values are different from one another. Any observed difference between means is tested for significance by dividing it by the standard error of the experiment which gives the value of 't'. For more than two sets of variables it was calculated by computer using the standard programme of L.S.D. While for the test of significance
between two sets of variables with known mean and standard deviations the 't' is calculated by the formula:

\[ t = \frac{\bar{X} - \bar{Y}}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}} \]

where \( \bar{X} \) = mean of one set of variables.

\( \bar{Y} \) = mean of another set of variables.

\( \sigma_1 \) and \( \sigma_2 \) = standard deviations of \( \bar{X} \) and \( \bar{Y} \) respectively.

\( n_1 \) and \( n_2 \) = means of \( \bar{X} \) and \( \bar{Y} \) respectively.