The possibility of improvement through induction of mutation in *Nigella sativa* L., a spice-yielding (Black cumin) plant of commercial importance, under the family Ranunculaceae, has been assessed and various cytological prerequisites as well as cytogenetical consequences of induced mutagenesis were explored in the present investigation. In this study observations were made on somatic and meiotic karyotypes, mitotic cell cycle, in vitro chromosomal variations, Giemsa C-banding, mutagenic responsiveness of the plant material, efficiency and effectiveness of the mutagens (X-ray and EMS), induced mutagenic variations in *M₁* and *M₂* generations, isolation of mutant lines and their cytogenetic behaviour, mode of inheritance of the mutant traits, quantitative variations in the mutant lines and correlation studies and path analysis of yield components in control and mutant plants. A concise account of the findings has been presented below.

1. *Nigella sativa* L. was found to contain 2n = 12 and n = 6 long to short-sized chromosomes in the somatic and meiotic complements. Barring the short telocentric pair, chromosomes
were nearly metacentric. Secondary constriction was located in two of the long pairs of chromosomes, but the longest pair did not bear such constriction. Karyotype formula should be $2n = 12 = 2A^m + 4ScB^m + 4C^m + 2t$.

Analysis of pachytene chromosomes depicted identical karyomorphology.

2. In vitro cytological studies revealed different types of irregularities. Elimination of chromosomes from the somatic complement was frequently observed in different cell lines, while the marker chromosomes (telocentrics) were found to be constantly present in the haploid, diploid, aneuploid and tetraploid cell lines; they appeared mostly in higher numbers than the other chromosomes of the complement. Variation in chromosome number has yielded production of polyploid, aneuploid and haploid cells.

3. Preceded with storage of air dried slides in Giemsa C-banding technique, presence of telomeric and centromeric bands were demonstrated in the chromosomes of *N. sativa*.

4. By labelling the cells morphologically with colchicine, duration of mitotic cycle was found to be 18 hours in the plant species.
5. For induction of mutations, seeds (1.5% moisture content) were treated with 4, 6, 8, 10, 20 and 30 Kr of X-rays and 0.5%, 0.75% and 1.0% EMS for 2 and 4 hours.

6. In the treated samples, germinality, survivability and rate of seedling growth have shown dose dependent decreasing trend. Similar responses were also noted in EMS treatments, but seedling growth was severely affected following 4 hours treatment with higher concentration. LD<sub>50</sub> was found to lie between 8 Kr and 10 Kr. It could not be ascertained in case of 2 hours treatment with EMS, but 50% lethality for seedling survivability in 4 hours treatment has been recorded at a slightly lower concentration than 0.5%.

7. Somatic cells of the control samples displayed rare occurrences of micronuclei and sticky bridges. The mitotic abnormalities encountered in the X-irradiated and EMS treated M<sub>1</sub> samples included fragments, diplochromosomes, ring configurations of chromosomes and aneuploid variation in chromosome number at metaphase; and multipolarity, laggards and fragments with or without bridges during anaphase. Predominant appearance of stickiness of chromosomes was marked in the treated samples. X-ray has been found to induce higher spectrum of mitotic anomalies than EMS.
8. In relation to control frequency of total mitotic anomalies increased, which has exhibited an increasing trend with an increase in the doses of X-irradiations and concentrations and durations of EMS treatments. On the contrary, mostly mitotic index has shown an inverse linear relationship with the employed doses and concentrations of the mutagens and with the induced cytological disturbances.

9. Meiosis was found to be normal in the control plants showing 6 bivalents at metaphase I and G+G separation in the anaphase I cells. In the M₁ treated samples, various types of meiotic irregularities were recorded, which included chromosomal fragments, univalents, multivalents, ring configuration of chromosomes, chromosomal grouping, non-orientation of univalents and bivalents and stickiness or clumping of chromosomes at metaphase I; and bridges with or without fragment and laggards at anaphase I. Besides, asynchronous condensation of chromosomes and duplication of number of chromosomes were also observed in few PMCs.

10. Pollen sterility in control plants of M₁ generation was found to be 2.2%. In the treated samples, sterility of both pollen and seed has shown dose dependent increasing trend exhibiting parallel relationship with the meiotic anomalies.
11. M1 characteristics on control and treated samples were studied. Some of the variants including chlorophyll deficient leaf chimaera and stem and floral anomalies appeared in M1 generation but did not recur in the M2.

12. Frequency of germination and survival of the M2 plants was recorded and the induced mutant types were screened from the very seedling stage to post-harvest condition. Frequency of mutations was found to be higher at the threshold doses of the treatments.

13. At the very seedling stage of the treated samples, 12 different types of chlorophyll mutations could be detected and they were described as, albina, xantha, lutea, chloroxantha, albescens, chlorina, chlorotica, coeruleovirens, margenata, viridis, albinoterminalis and xantha-terminalis. Besides margenata, chloroxantha, coeruleovirens and viridis, all other chlorophyll mutations failed to survive beyond their seedling stage. Excepting viridis, no other chlorophyll mutants could, however, be recovered in the subsequent generations. Both viridis and chloroxantha types of chlorophyll mutations were found to be controlled by two pairs of recessive genes.
14. Excluding chlorophyll mutations, 13 different other mutant types could be isolated in the M2 generation; of which lax branching, feathery leaf, bushy, male sterile, crumpled leaf, dwarf, early flowering, prostrate and brown seed-coat mutant types were viable, while cup-like, needle leaf and cotyledonary leaf mutants were non-viable.

15. From F2 segregation and crossing experiments performed between normal and mutant plants the lax branching trait, feathery leaf condition, bushyness, male sterility and dwarfness were found to be controlled by single pair of recessive genes.

16. Mutagenic effectiveness and efficiency have been calculated taking into account of the overall mutation frequency. Both mutagenic effectiveness and mutagenic efficiency have exhibited dose dependent decreasing trend.

17. Variability in polygenic traits of M2 lines were assessed and frequency distribution for different phenotypic characters were shown.
Occurrence of cytomixis was detected in the $M_2$ generation of a *lex* branching mutant obtained through EMS treatment. Presence of cytomixis was restricted only in the prophase I cells of meiotic division. Transfer of nuclear materials from one PMC to the adjacent PMCs took place through cytoplasmic links and it occurred at random. Cytomixis resulted in the formation of aneuploid (hypo- and hyperdiploid) and polyploid PMCs. Normal seed setting was not affected in the *lex* branching mutant having only 6.74% pollen sterility. From the selfed progeny ($M_3$) of the mutant, two aneuploids were detected (9.65%); one of which was a trisomic, while the other aneuploid (morphologically aberrant too) demonstrated meiotic instability within the same microsporophyll indicating possibility of chromosomal mosaicism.

Phenotypically the trisomic plant was weak with slender stem and drooping laminae. The extra chromosome in the trisomic plant was present mostly as an univalent and rarely as trivalent. The plant was completely seed sterile and has arisen possibly as a chance occurrence through union of normal and aneuploid gametes, the latter being formed as the consequence of cytomixis.
19. A feathery leaf mutant was detected in the M$_2$ population following 2 hours treatment with 0.5% EMS. In the selfed progeny of the M$_2$ mutant four extremely dwarf plants were recovered having identical leaf phenotype like their progenitor. Cytogenetically the parent (M$_2$) and the dwarf mutant (M$_3$) were characterised by the presence of paired fragments and multivalents respectively. The dwarf mutant has been designated as "telescopic mutant". Multivalent association in the telescopic mutant has arisen possibly due to deletion followed by chromosomal interchanged in the M$_2$ plant. Among the four such telescopic mutant plants raised in the selfed progeny (M$_4$) of the telescopic plants, one showed prevalence of ring like multivalents (associations), while the others forming normal bivalents. The M$_4$ cytogenetically marked telescopic mutant was found to be semi-sterile. Origin of the telescopic mutant line has been ascribed to deficiency of genes as an outcome of chromosomal deletion in the parent.

20. A bushy mutant with desynaptic behaviour of chromosomes has been recovered from the M$_2$ population following 2 hours treatment with 0.5% EMS. A single pair of recessive genes (bu/bu) has been ascribed for bushyness and the mutant bred true in the subsequent generations. The bushy
mutant plant could always be characterized by their delayed germination, flowering and maturity, high frequency of sterile pollen, poor seed setting and desynaptic behaviour of chromosomes. Possibly, the mutant gene has shown pleiotropic effect or the traits were closely linked with the bushy phenotype.

21. A EMS-induced male sterile mutant with marked foliage characteristic and synchronous flowering has been recovered. Crossing experiments performed with normal fertile pollen parent demonstrated existence of partial female sterility in the mutant. Meiotically the mutant was normal but failed to produce any pollen grain due to post-tetrad developmental disturbances. A recessive factor (ms ms) has been attributed for male sterility.

22. Findings on different plant characteristics, namely, frequency and rate of germination, survival and post-harvest observations were recorded in control and different mutant lines (lex-branched, feathery leaf, bushy, early flowering, brown seed-coat, viridis, dwarf and prostrate). Further, sterility of flower, pollen and seed was assessed in the control and mutant plants during M₂, M₃ and M₄ generations.
23. Phenotypic variables have been studied in control plants under uniform field trial conditions for four successive years (1979-80, 1980-81, 1981-82 and 1982-83) and the result analysed through analysis of variance revealed significant mean differences for different traits for different years.

24. Analysis of variance of different quantitative traits has been made in pure lines of controls and in six true breeding M₄ mutants (lax branching, feathery leaf, bushy, early flowering, brown seed-coat and viridis), which demonstrated significant variations among the samples for most of the attributes, thus the possibility of scope of improvement of the plant types following repeated selection and hybridization was indicated.

25. The phenotypic correlation coefficients between yield and its related components (plant height, number of primary branches per plant, total capsules per plant and filled seeds per capsule) were studied and the components of correlations were computed through path analysis for determining the nature of direct and indirect effects of yield contributing attributes in control and six mutant plant types (lax branching, feathery leaf, bushy,
early flowering, brown seed-coat and viridis). Results of path analysis definitely indicates that total number of capsules per plant is the most reliable trait contributing towards seed yield. Obviously, total number of capsule per plant has been found to be the important selection index for bringing effective improvement in the plant types.