Review of literature in *N. sativa* is documented on the related aspects on which present investigation has been performed.

**POLLINATION BIOLOGY**

Self pollinated; onset of the male stage stamen stand erect, curved outwards one by one, roughly in whorls and strictly reflecting the order of initiation, pollen grains released when anthers reach a horizontal position; male phase initiated a few days before the stigmas became receptive and lasted for five days; anther receptivity occurred between 8.00 h to 13.00 h for one day only, male and female stages synchronized on the last day of the flowering; weight of pollen 0.064 mg/flower whereas the volume of nectar 0.13 µl (Abu-Hammour 2008); empty anthers curved up; pollinated stigma erect and made an angle of 180º with the ovary; style and anther length nearly equal 1.73 cm; pollinator honey bee, one bee per flower, visited in morning around 7.00 a.m.; high temperature effect fertilization success by affecting stigma receptivity and accelerating ovule degeneration (Postweiler *et al.* 1985).

**MITOTIC AND MEIOTIC CONSEQUENCES OF MUTAGEN AND CHEMICAL TREATMENTS:**

Biswas and Bhattacharyaya (1975) studied the effect of some mutagens and chemicals like maleic hydrazide (MH), acridine orange (AO), ethyl urethane and ethylene-diamine-tetracetic acid (EDTA) at variable concentrations and durations on the root tip mitosis of the species. The chemicals induced cytological aberrations viz., fragments, laggards, micronuclei, grouping and stickiness of chromosomes and reduced mitotic index in prolonged treatments and in higher concentrations. It was suggested that the chemicals possibly affected nucleic acid synthesis in differential manner which ultimately causes hazards in replication thereby inducing chromosome breakage.

Kumar and Nizam (1976) studied induced somatic pairing of homologous chromosomes from root tip mitosis following the treatment with mitomycin C. It was observed that the homologous chromosomes become juxtaposed to each other with remarkable regularity in the prometaphase cells following treatment for 40 minutes, whereas the untreated cells showed no such associations. It was presumed that these
movements may be due to kinetochore activity which normally causes congregation of chromosomes towards the equatorial plate of the spindle but which does not occur contemporaneously in all chromosomes. In view of the observations, it was suggested that kinetochores were responsible for placing homologues near each other and stickiness was attributed to be a factor for association of homologous chromosomes.

Chand (1980) reported that pentachlorophenol (PCP) inhibited mitosis in shorter duration of treatments and cytological abnormalities were formed. Incorporation studies revealed that PCP inhibited DNA synthesis. The chemical was found to affect nuclear membrane cycle, chromosome division cycle, spindle organization and chromosome movements, condensation and spiralization of chromosomes and DNA and protein synthesis.

Mandal and Basu (1978) studied X-ray induced chromosomal aberration from leaf meristems, pollen mother cells and endosperm of *N. sativa*. In all cases the aberration percentage increased with increase in doses and decreased with time lapse from two hours to twenty four hours after irradiation. It was suggested that endosperm being the most resistant tissue though it had the largest ICV (Interphase Chromosomal Volume) value. Kumar and Nizam (1978) assessed the effect of X-rays on dry and pre-soaked seeds of *N. sativa* and noted that the frequency of mitotic and meiotic aberrations in the pre-soaked seeds was higher than that in the dry seeds following irradiation. The aberrations encountered were mostly related to spindle organization and formation of dicentrics, rings, micronuclei and acentric fragments. Similar types of radio-mimetic anomalies were also studied from EMS induced *N. sativa* and *N. damascena* (Datta and Biswas 1981). Datta and Biswas (1983) reported X-ray sensitivity of dry seeds of *N. sativa*, and LD$_{50}$ was found to lie between 8kR and 10kR. X-rays were found to induce chromosomal breakages at mitosis, physiological damages affecting seedling growth and germination frequency under Petriplate conditions, meiotic abnormalities and pollen sterilities. Datta *et al.* (1986) also suggested gamma irradiation sensitivity of the species following exposure of dry seeds. LD$_{50}$ was reported to be in between 20kR and 30kR and beyond 30kR irradiation was lethal to the species.
Rang and Datta (1998) exposed dry, pre-soaked (12 hours in distilled water), totally dehydrated and stored (one year six months stored under desiccation; one season stored seed) seed samples (moisture content: 7.5%) of *N. sativa* to gamma irradiations (5, 10 and 20 kR doses) and also that some amount of the dry irradiated materials were treated with ethyl methane sulphonate (EMS) and hydrogen peroxide (H\(_2\)O\(_2\)) for six hours at 0.25 percent to evaluate the cytogenetic changes that might occur due to gamma-irradiation influenced by the physical and chemical factors. Assessment of radio-sensitivity was made from attributes like seed germination, rate of seedling growth, mitotic index, frequency and spectrum of chromosomal aberrations in root tip cells and pollen and seed sterilities of M\(_1\) plants as well as M\(_2\) mutation (macromutants) frequency. Results indicated that the factors (physical and combined treatments) have influenced gamma radiation sensitivity in inducing cytogenetical and genetical changes along with M\(_2\) mutation frequency.

**INDUCED MUTAGENESIS**

**Variants in M\(_1\) generation**

Datta and Biswas (1985) reported bifurcation, trifurcation, twisting, unbranched and twining nature of stem in M\(_1\) generation following X-ray irradiations and EMS treatments. Apart from stem anomalies some floral abnormalities were also recorded like adnation of sepals, elongated and strap shaped petals, two gynoecium in the same flower and presence of bract like structures. However, the abnormalities studied at M\(_1\) did not recurred at M\(_2\) and such non-inheritable changes were considered due to somatic mutation.

**Macromutants and their inheritance pattern**

Kumar and Nizam (1983) induced (X-rays and gamma rays) few viable mutants such as multicolor capsular fruits and color fruit coat with ornamentations including mutation affecting branching pattern and fertility at M\(_2\). Datta and Biswas (1985) induced (X-ray and EMS) several mutants in relation to normal trait. Threshold doses were efficient and 0.5% EMS, 2 hours treatment was reported to be the best among all the treated doses. Chlorophyll mutations occurred predominantly than other types and
among them *viridis* and *chloroxantha* were viable types and found to be controlled by two pairs of recessive genes.

Mitra and Bhowmick (1997) induced ten different types of chlorophyll mutation in two cultivars of *N. sativa* following treatments with gamma irradiation and EMS. Higher doses of gamma-rays and lower concentration and duration of EMS were reported to be most efficient. Mitra and Bhoumick (1998) studied the mutagenic effects (gamma irradiation and EMS) of some biological parameters in M\textsubscript{1} generation and suggested that gamma irradiations were more effective than EMS and the cultivar KS-1 was more sensitive to mutagens under the tested doses and concentrations.

Rang and Datta (2001) spotted five dark reddish brown (color code - 3/2), one yellowish brown (5/4) and one peach (512/1) color seeded plants at M\textsubscript{2} following different treatments of gamma irradiations and EMS. Mutation frequency of dark reddish brown color, yellowish brown color and bicolor was estimated to be 1.92, 0.055 and 0.54 per cent respectively. Following crossing experiments the genotypes BBPP, b\textsuperscript{dr}b\textsuperscript{dr}PP, b\textsuperscript{y}b\textsuperscript{y}PP and bbpp were assigned for black, dark reddish brown, yellowish brown and bicolour seed-coat plant types. Datta and Rang (2001) reported a viable *chloroxantha* mutant in black cumin.

**Polygenic mutation**

Datta and Biswas (1993) analyzed variations for quantitative characteristics and reported that mutation was random in nature releasing variations in both positive and negative directions.

**Biochemical studies on induced mutants**

Datta *et al*. (1987) performed electrophoretic characterization (SDS-PAGE) of seed protein of control and mutant plant types and were of opinion that band profile can be used as an additional parameter to supplement cytogenetical data for proper understanding of the genetic variations released through induced mutagenesis.

**MALE STERILITY**

Datta and Biswas (1984) isolated a *male sterile* mutant possessing dark green, thick and leather like pinnae of the leaves associated with synchronous flowering. The
mutant showed complete indehiscence of the anthers and lacked pollen grain formation at pollination. Datta and Rang (1998) isolated a male sterile mutant with lax pinnae along with yellowish green pinnae in the shoot apex of the primary axis. The mutant showed desynapsis and were pollenless at anthesis. Datta and Saha (2001) identified few male sterile lines following gamma irradiations at different doses. The mutants were with detectable phenotypic marker trait(s) and showed pollen size variations, agglutination and degeneration as well as 100\% sterile pollen grain formation. The male sterile mutants were considered as non-structural nuclear type.

**GENETIC VARIATION**

Datta (1984) from correlation and path coefficient analysis of different quantitative traits was of opinion that capsule/plant has been the most important trait for selection and crop improvement in black cumin. Iqbal et al. (2009) studied 34 accessions with 2 check genotypes of black cumin for assessment of mineral nutrients. High variation was recorded for Fe, Ca, Cu, Mg, Pb, Zn, Co, Mn, Na, P, B, K and N among the genotypes suggesting sample selection based on the composition of mineral nutrients. Correlation studies revealed significant association between Cu and Ca and between Mg and Ca and Mg and Cu. Based on principle component analysis (PCA) six clusters were observed and it was suggested that the genotypes may be utilized in various combinations for genetic improvement of the species. Iqbal et al. (2010) recorded genetic variation for plant height, days to first flower, days to 50\% flowers, days to maturity, biomass, capsule weight, yield, seed weight and harvest index while studying 31 genotypes under field conditions with 3 replications. Three accessions namely MP00023, MP00111 and MP00120 were found better for more than one character and are expected to be a potential for improvement of *N. sativa*.

**MOLECULAR GENETICS**

Al-Huqail and Al-Saad (2010) performed DNA fingerprinting in 4 accessions from Saudi Arabia, Ethiopia, Egypt and Syria with an objective of genotypic characterization between/among black cumin taxa. Inter Simple Sequence Repeat
(ISSR) method was employed in the PCR technique to detect genetic polymorphism. The scored bands of the DNA fingerprints (17 primers representing 3 types of intermicrosatellites – di, tri and tetra of short tandem repeats) were 108 in Saudi Arabia, 106 in Ethiopia, 100 in Egypt and 81 in Syria and the percentage of dissimilarity was computed to be 21.5-36.3%. Twenty four genes representing 24 different enzymes and isozymes were selected and scanned via PCR technique using suitable SSR primers and the obtained results showed few changes in the genetic structure of some of the genes. Iqbal et al. (2011) carried out investigation to explore genotype specific fingerprinting of 32 germplasms based on randomly amplified polymorphic DNA markers. From 58 random primers used, 15 primers generated 249 reproducible and scorable amplification products across all the genotypes, out of which 164 (66.0%) fragments were polymorphic revealing a high level of polymorphism among the genotypes. The proportion of common bands was low (34.0%). In 13 genotypes, 27 bands of different masses (kilo bases) were recorded and were considered specific. The specific/amplified PCR products were reported to be used as molecular markers for identification of genetic polymorphism which was validated from UPGMA and PCA.

From the perusal of literature it seems that ‘plant type’ mutations were induced in the species but significant mutants namely synchronous flowering, non-shattering capsule among others are still lacking, which will be of economic worth for the plant species. Further, an attempt has been taken to induce and maintain genetic male sterile line(s) and a comprehensive analysis of such plant type from cytogenetical and molecular point of view may provide better insight on the phenomenon. Thus, the present investigation has been designed on induced mutagenesis of \textit{N. sativa} using 6 different mutagens. Present study will also reflect the potentiality of the mutagens assessed over a plant species.