MITOTIC AND MEIOTIC CONSEQUENCES OF GAMMA IRRADIATIONS ON DRY SEEDS OF *NIGELLA SATIVA* L. (BLACK CUMIN)

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Abstract: Dry seeds (moisture content :19.04%) of *Nigella sativa* L. (Family: Rananculaceae; common name - black cumin, spice of commerce) were gamma irradiated (50, 100,150, and 200 Gray doses) and M_1 (germination frequency, seedling length, lethality, injury, mitotic index, mitotic aberration frequency; meiotic abnormalities, pollen fertility and seed sterility) parameter and M_2 mutation (macromutants) frequency were studied with an objective to assess mutagenic sensitivity as a pre-requisite for mutation breeding experiment. LD_{50} was found to be between 50 Gy and 100 Gy. Results obtained are discussed.

Keywords: Gamma irradiations, M_1 parameters, mitotic & meiotic aberrations, *Nigella sativa.*

INTRODUCTION

Nigella sativa L. (Family: Rananculaceae; common name - black cumin,) yields spice of commerce apart from possessing insecticidal, antimicrobial (Morsi 2000), antioxidant (Burtis and Buear 2000) and anticancerous (Badary 1999) properties. Besides its potential significant commercial importance, the species is a good material for cytological and cytogenetical studies (Saha and Datta 2008). Cytological and cytogenetical consequences of X-irradiations and EMS (Datta and Biswas 1983, Datta *et. al.* 1986) and gamma irradiations (Rang and Datta 1998, Saha and Datta 2002) were reported earlier in the species. Present communication is an additional endeavor to describe mitotic and meiotic consequences arising out of gamma irradiations on the dry seeds of *N. sativa*, prerequisite for any mutagenesis experiment, with an objective to isolate potential mutants aiming to crop improvement. Aberrations involving chromosome breakages and their genetic outcome form an integral part of the studies on radiation genetics.

MATERIAL METHODS

Plant Material

Mother seed stock of *Nigella sativa* L. obtained from Sutton's and Sons, Kolkata (moisture content: 19.04 %; seed size; 0.27mm ± 1.25 × 0.16mm ± 0.69; 100 seed weight: 0.21gm ± 0.003) is used as material source for mutagenic treatment.

Mutagenic Treatment and Raising M_1 and M_2 Generations

Dry seeds of *Nigella sativa* were gamma irradiated (^{60}Cobalt source) with 50,100, 150 and 200 Gray (Gy) doses from Saha Institute of Nuclear Physics, Salt Lake, Kolkata. Each treatment comprised of 100 seeds. Fifty seeds from each treatment along with untreated control were given in petriplates lined with moist filter paper (room temperature:24°C±1°C) to assess germination (bursting of seed coat and emergence of radical) and seedling length (on 10th day of treatment; 15 seedlings were randomly studied; lethality and injury were estimated from relative reduction in germination and seedling growth respectively in treatments in relation to control – Konzak *et al*. 1965); while, the other lot (50 seeds in each treatment along with control) was transferred to experimental field plots of Department of Botany, University of Kalyani (Latitude22°50' to 24°11' N, longitude 88°09' to 88°48' E; soil- 9.9% sand, 2.9% silt and 87.2% clay; organic carbon-3.25%; soil pH 6.87) to raise M_1 plant population (late November to early April). Selfed seeds (first formed 2 to 3 flowers of each plant and in each treatment were bagged) of each surviving M_1 plant were harvested separately and M_2 plants (spacing 15 cm between plants and 25 cm between lines) were raised as plant to row progenies. The treated as well as control plants populations were carefully screened for macromutants throughout the growth period in the field and the frequency of the mutants was assessed as per 100 plants (Gaul 1964).

Cytological Studies

For mitotic study, healthy root tips (1.0 to 1.5 cm in length) from control and treated samples (4 to 5 root-tips were collected randomly from each treatment) were fixed (acetic alcohol 1:3 for overnight), stained (orcein- HCl mixture 9:1; gently warmed in the solution for 8 - 10 min.) and squashed (45% acetic acid) before observation (two slides were scored for each treatment) under the microscope. Frequency of the mitotic abnormalities was determined from total number of dividing cells and that of micronuclei, giant cells and nuclear shape abnormalities from resting cells.

Meiotic observations were made from flower buds (3 to 5 buds were fixed from randomly selected plants from each treatment including control) fixed in propiono-alcohol (1:3) between 5:30 am to 6:30 am and preserved in 70% alcohol. Pollen mother cells (PMCs) were stained in 1% propiono-carmine solution. Data were scored from metaphase I (MI)

and anaphase I (AI) cells. Photomicrographs of cytological preparations were taken from temporary preparations.

Sterilities

Sterility of pollen grains (pollen grains were squeezed out of mature anthers) was studied by staining pollen in 1% aceto-carmine and unstained or partially stained pollen grains were classified as sterile (Marks 1954). Seed sterility was represented as percentage of reduction in seed weight in treatments in relation to control.

RESULT AND DISCUSSION

Results of M_1 and M_2 attributes are presented in table 1. Parameters like seed germination, seedling growth and mitotic abnormalities including mitotic index (studied under control conditions) and pollen fertility as well as seed sterility were assessed in M_1 generation in comparison to control to estimate mutagenic sensitivity of the species to gamma irradiations. Assessment of mutagenic sensitivity is a pre requisite in any mutation breeding programme. Frequency of seed germination, seedling growth and total mitotic abnormalities estimated from dividing cells demonstrated dose dependent relationship. The percentage of lethality and injury enhanced in relation to the doses of treatments. Reduction in germination frequency and seedling growth due to irradiations was attributed to the nature and extent of chromosome aberrations occurring in the cells (Sax 1942, Read 1959, Evans and Sparrow 1961, Datta and Biswas 1983, Datta *et. al.* 1986). Evans (1965) considered blockage of cells into mitosis as the most important cellular event after mutagenic treatments, which results in cessation of growth. Gunkel and Sparrow (1954) were of opinion that retardation in growth due to mutagen treatments may be primarily due to destruction of auxin of meristematic cells as well as due to physiological and biochemical disturbances. In the present investigation, LD_{50} seem to lie between 50 Gy and 100 Gy as evidenced from lethality.

Mitotic abnormalities (Figs. 1-12) recorded in gamma irradiated samples (control- only sticky bridge formation) were fragments, sticky and ring configuration of chromosomes (Fig. 2) and diplochromosomes at metaphase and laggards and bridges with or without fragments at anaphase (Figs. 3-7). Bridges (notably single, paired, criss-cross, inter-locked and broken) were noted predominantly in higher doses of treatments. Paired fragments of identical sizes appeared in anaphase cells of 200 Gy (Fig. 7) treatment. Micronuclei (1 to 4; variable in sizes; condensed as well as uncondensed - Figs. 8-9), giant cell (Fig. 10) and nuclear shape irregularities (Fig. 11) were observed in resting cells. Rare often cell fusion (150 Gy) was recorded at metaphase (Fig. 12). Bridges were formed due to breakages and re-union; while, micronuclei appeared as the consequence of fragmentation in chromosomes in dividing cells and these anomalies were interrelated between/among themselves (micronuclei-x, fragment-y, bridge-z; correlation analysis: $r_{xy}=0.97$, $p<0.05$; $r_{xz}=0.82$, $p<0.05$; $r_{yz}=0.83$, $p<0.05$, DF 4 in all cases). Occurrence of giant cells may be the outcome of physiological disturbances.

Studies of meiosis forms an integral part in induced mutagenesis experiment as changes occurring in gametic cells may be reflected genetically in M_2 generation. PMC revealed $2n = 12$ chromosomes in control as well as in treatments (Figs. 13-21). Predominant association noted in the plant types were 6II formation (Figs. 13-14). Average chromosome association per cell documented enhanced frequency of univalents (Figs. 15-17) and formation of quadrivalents in treatments as compared to control. Univalents were possibly the outcome of pairing defects; while, formation of quadrivalents indicated structural alterations in chromosomes. Enhanced frequency of quadrivalent formation in higher doses suggested the possibility of screening translocation heterozygotes. Quadrivalents (Figs. 19-21) were mostly of ring configuration (89.79%) and rarely chain (10.20%). Most of the ring quadrivalents were of adjacent (Figs. 19-20) orientation (63.64%); while the rest were alternate (34.09%) and rare often non co-oriented (Fig. 21) (2.27%). A PMC (Fig. 18) at 200Gy was observed to posses 6II + two nearly identical sized (2.39 µm and 2.59 µm) fragments (1.21%). Occurrence of paired fragments at M_1 possibly suggested localized breakages in the chromosomes with sub-terminal constriction. Moutschen (1968) observed localized breakage in or near the end parts of the chromosome of *N. damascena* following irradiations with ionizing rays. On the contrary, paired identical sized fragments (5.38 µm) noted at AI seems to be the breakage in one of the two telocentric chromosomes (one telocentric is marked intact at one pole – Fig. 23). Ghosh and Datta (2006) reported a marker telocentric pair in the karyotype of *N. sativa*. AI cells in treatments (rare often unequal separation of chromosomes, fragments (Fig. 23) and bridges with an accompanying fragment (Fig. 24) were noted) were mostly balanced (6:6) cytologically as in control (Fig. 22) but pollen fertility was reduced in treatments than control. High seed sterility in treatments may be the outcome of pollen sterility. Results therefore indicated that gamma irradiations have induced cytological abnormalities thereby resulting in genetical changes which reflected possibly in the occurrence of M_2 (macromutant frequency is variable; four types of mutants scored due to low turn over of M_2 plants; type of macromutants: *male sterile*, *thick stem*, *narrow pinnae*, *slender stem*, *elongated rachis*, *lax branching* and *delayed shattering of pods* – data unpublished) mutants.

FIGURE LEGENDS

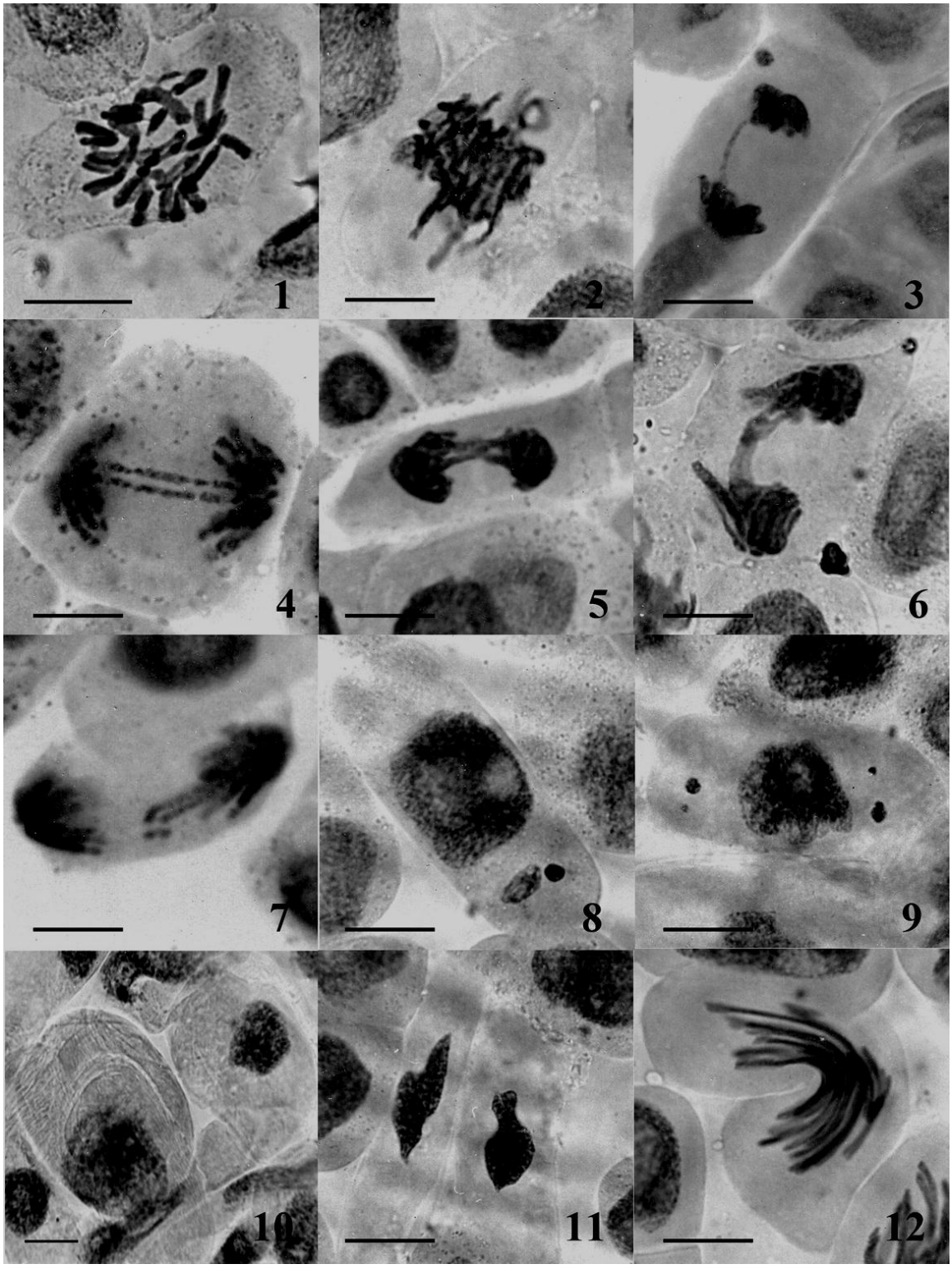

Figure plate I (1 - 12) showing mitotic consequences (**1-2:** metaphase; **3-7:** anaphase; **8-11:** resting cell) of gamma irradiations in *Nigella sativa*. **(Fig. 1)** 2*n* = 12 chromosome; **(2)** ring configuration of chromosomes; **(3)** single bridge and a fragment; **(4)** paired bridge; **(5)** criss-cross bridge; **(6)** interlocked bridge; **(7)** broken paired bridge with 2 identical sized fragments; **(8)** two (condensed as well as uncondensed) unequal sized micronuclei; **(9)** four unequal sized micronuclei; **(10)** giant cell; **(11)** irregular shaped nuclei; **(12)** fusion of two cells. Scale bar = 20 µm.

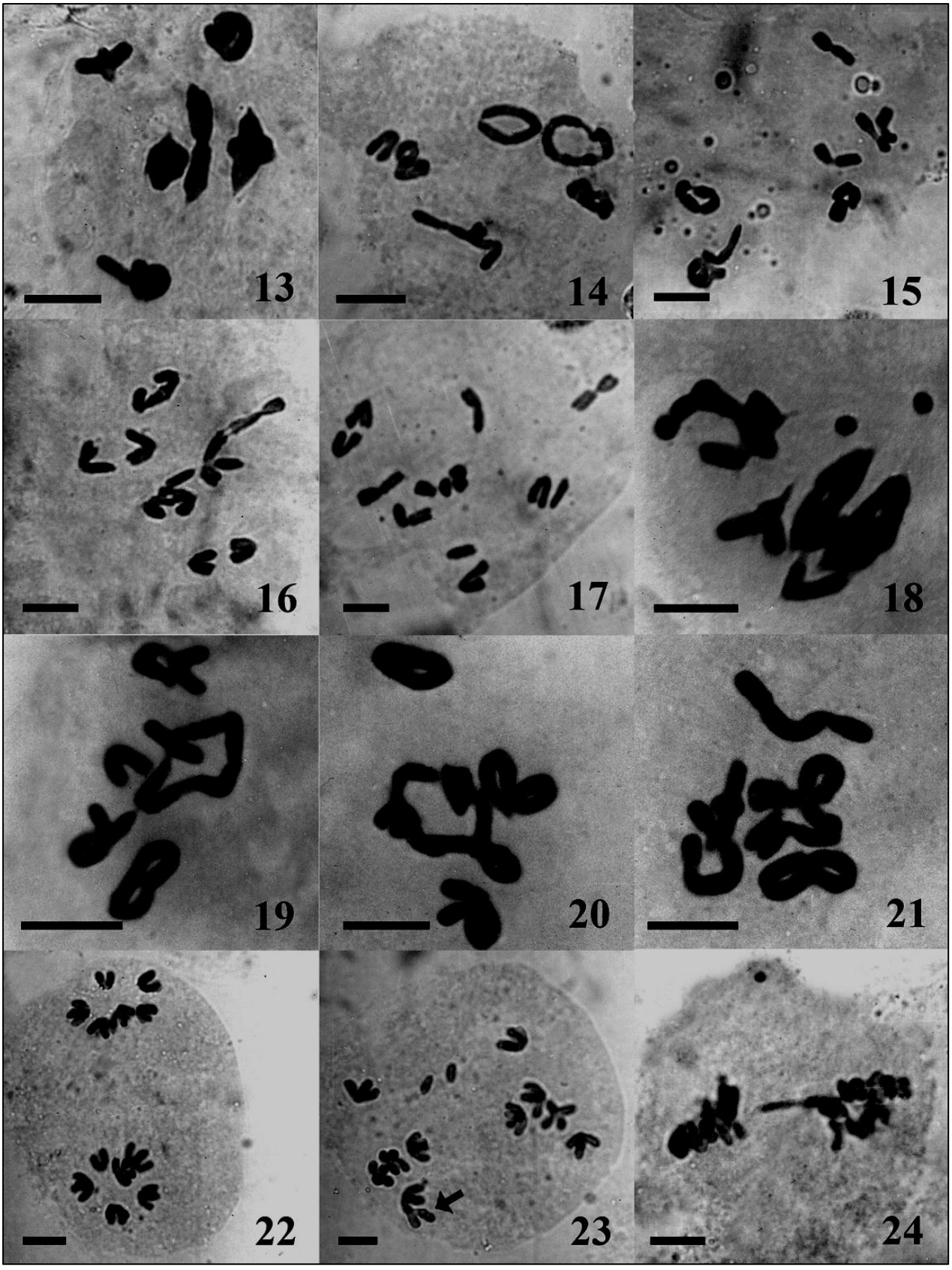

Figure plate II (13 - 24) showing meiotic configurations in control **(Fig. 13)** and irradiated samples **(14 - 24)** at MI **(13 - 21)** and AI **(22 - 24)** of *Nigella sativa*. **(Figs. 13 - 14)** 6II; **(15)** 4II + 4I; **(16)** 2II + 8I; **(17)** 12II; **(18)** 6II + 2 identical sized fragments; **(19 - 21)** 1IV + 4II; **(22)** 6:6 separation of chromosomes; **(23)** 2 equal sized lagging fragments; (**24)** dicentric chromatid bridge with an acentric fragment. Scale bar = 10 µm.

Table 1 M_1 and M_2 attributes in control and gamma-irradiated samples of *N. sativa.*

Attributes	Control	Radiation doses (Gy)			
		50	100	150	200
Germination (%)	82.00	40.00	32.0	28.00	36.00
Seedling length (mm)	52.6±2.36	40.2±5.42	33.7±4.31	30.4±6.62	17.7±2.36
Lethality(% of control)	-	43.91	60.98	65.59	56.10
Injury (% of control)	-	23.58	35.94	43.21	66.35
Mitotic index (%)	7.57	2.16	5.13	4.17	4.93
Aberrant dividing cells (%)	0.24	46.51	38.09	70.33	76.43
Frequency of cells with fragments (%)	0.00	0.023	0.016	0.025	0.030
Frequency of cells with bridges (%)	0.00	0.016	0.032	0.025	0.071
Micronuclei (%)	0.00	0.357	0.006	0.013	0.016
Mean association at MI	5.73II+0.53I	5.76II+0.48I	4.51II+2.97I	0.04IV+4.04II+3.79I	0.59IV+4.78II+0.29I
MI cells scored	109	134	78	114	82
Equal AI chromosome separation (%)	94.11	89.09	87.27	86.44	88.88
AI cells studied	34	55	110	59	36
Pollen fertility (%)	88.97	53.18	54.90	50.69	51.29
Pollen grains analyzed	1079	1036	1051	649	655
Seed sterility (%)	-	33.75	26.25	23.75	52.50
M_2 mutation frequency (%)	0.00	11.00	8.13	15.07	1.33
Plants scored	77	200	160	73	75

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REFERENCES

Badary, O.A. (1999). Thymoquinone attenuates ifosfamide-induced Fanconi syndrome in rats and enhances its antitumour activity in mice. *Journal of Ethnopharmacology*, **67:** 135-142.

Burtis, M. and Bucar, F. (2000). Antioxidant activity of *Nigella sativa* essential oil. *Phytotherapy Research*, **14:** 323-328.

Datta, A.K. and Biswas, A.K. (1983). X-rays sensitivity in *Nigella sativa* L. *Cytologia*, **48:** 293-303.

Datta, A.K.; Biswas, A.K. and Sen, S. (1986). gamma radiation sensitivity in *Nigella sativa* L. *Cytologia*, **51:** 609-615.

Evans, H.J. and Sparrow, A.H. (1961). Nuclear factors effecting radiosensitivity II. Dependence on nuclear and chromosome structure and organization. *Brookhaven Symposium Biology*, **14:** 101-127.

Evans, H.J. (1965). Effects of radiation on meristamatic cells. *Radiation Botany*, **5:** 171-182.

Gaul, H. (1964). Mutations in plant breeding. *Radiation Botany*, **4**: 155-232.

Ghosh, A. and Datta, A.K. (2006). Karyotyping of *Nigella sativa* L. and *Nigella damascena* L. by Image Analysing System. *Cytologia*, **71:** 1-4.

Gunckel, J.E. and Sparrow, A.H. (1954). Aberrant growth in plants induced by ionizing radiations. *Brookhaven Symposium Biology*, **6:** 252-277.

Konzak, C.F.; Nilan, R.A.; Wagner, J. and Foster, R.J. (1965). Efficient chemical mutagenesis. *Radiation Botany*, **5:** 49-85.

Marks, G.E. (1954). An aceto-carmine glycerol jelly for use in pollen-fertility counts. *Biotechnic and Histochemistry*, **29:** 277-278.

Moutschen, J.H. (1968). Some implication of radio-induced structural changes of chromosome in *N. damascena*. *Nucleus*, **11:** 177-188.

Morsi, N.M. (2000). Antimicrobial effects of crude extracts of *Nigella sativa* on multiple antibiotics – resistant bacteria. *Acta Microbiologica Polonica*, **49:** 65-74.

Read, J. (1959). Radiation Biology of *Vicia faba* in Relation to General Problem. *Black-well Scientific publications, Oxford*, pp. 270.

Rang, S.K. and Datta, A.K. (1998). Influence of some physical and chemical factors on gamma radiation sensitivity in *Nigella sativa* L. (black cumin). *Journal of National Botanical Society*, **52:** 17-22.

Saha, A. and Datta, A.K. (2002). Gamma-rats induced reciprocal translocation in black cumin (*Nigella sativa* L.). *Cytologia*, **67:** 389-396.

Saha, A. and Datta, A.K. (2008). Black cumin (*Nigella sativa* L.) – A model plant for cytogenetical studies. *Plant Archives*, **8:** 1-5.

Sax, K. (1942). The mechanisms of X-ray effects on cells. *The Journal of General Physiology*, **25:** 533-537.