Effectivity of copper and cadmium sulphide nanoparticles in mitotic and meiotic cells of *Nigella sativa* L. (black cumin) — can nanoparticles act as mutagenic agents?

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**ABSTRACT**

The present investigation deals with wet chemical preparation and characterisation of copper (Cu) and cadmium sulphide (CdS) nanoparticles (NPs) (using UV–visible spectra, Fourier transform infra-red scattering, X-ray diffraction, dynamic light scattering, field emission scanning electron microscopy and transmission electron microscopy) and their effectivity on mitotic and meiotic cells of *Nigella sativa* L. (Ranunculaceae) in comparison to ethyl methanesulphonate (EMS) and gamma irradiations. The objective of the study is to foresee whether Cu- and CdS-NPs can induce similar type of chromosomal aberrations as that of EMS and gamma irradiations, or not. Dry seeds of *N. sativa* (2n = 12) are exposed to Cu- and CdS-NPs (0.25, 0.50 and 1.0 μg/ml; 3 and 6 h), EMS (0.25, 0.50 and 1.0%; 3 and 6 h) and doses of gamma irradiations (25, 50, 100, 200 and 300 Gy). Cu-NPs (range: 25.7 to 120.4 nm; 33.2 nm ± 9.6) and CdS-NPs (range: 29.4 to 115.7 nm; 37.8 nm ± 10.7) are both cubical to spherical in shape. NPs are found to induce similar responses as that of the studied conventional mutagens, in relation to physiological and chromosomal (mitotic and meiotic) attributes. Uptake of Cu- and CdS-NPs in seedlings is also studied using atomic absorption spectroscopy. Results suggest that Cu- and CdS-NPs can act as mutagenic agent, a pioneer report of its kind.

**1. Introduction**

Nanotechnology has global significance due to its wide application in industry, medicine, biotechnology, agriculture, different aspect of life sciences, among others.[1—12] Nanoparticles (NPs — at least one dimension ranging between 1 and 100 nm — [1]) possess unique physico-chemical properties,[9,10] which can be drastically modified compared to bulk materials.[13] They are frequently released in the environment by natural processes and anthropogenic activities [4,14—18] effecting biological systems through cellular uptake and interactions. It raises debate in scientific community regarding risks and benefits associated to it.[19] NPs are reported to exert both advantageous [20—23] and adverse [24—31] effects on higher plants. Physico-chemical properties of synthesised NPs, their size and surface area and doses employed are determining criteria for their bioactivity. [9,10,32]
NPs are reported to induce cytotoxic and genotoxic effects on mitotic cells of different plant species.\cite{7,8,32} Till date, cytotoxic effects of NPs on meiotic cells are rather lacking. Effect on meiotic cells of plant species are expected to bring about genetical changes. Halder et al. \cite{11,12} reported that copper (Cu) and cadmium sulphide (CdS) NPs induce stable and heritable phenotypic changes in *Macrotyloma uniflorum* (Lam.) Verdc (Family: Leguminosae). Such findings trigger the necessity to acquire further scientific knowledge from plant system on cytogenetical aspects relating to NP studies.

The present investigation describes the effects of synthesised Cu- (Cu\(^{2+}\) — metallic NPs, used as microfertilisers and pesticides \cite{43,44}) and CdS-NPs (semiconductor in nature, also known as quantum dots, narrow band gap of 2.5 eV \cite{45}; used widely in biology, medicine, technology and telecommunication \cite{46–48}) on mitotic and meiotic cells of *Nigella sativa* L. (common name: black cumin; Family: Ranunculaceae; spice-yielding plant of commerce possessing immense therapeutic uses \cite{49} and cytological novelty \cite{50}), in comparison to conventional mutagens namely, ethyl methanesulphonate (EMS) and gamma irradiations. The objective of the work is to foresee whether the synthesised NPs can induce similar type of chromosomal aberrations as that of the studied conventional mutagens or not. If NPs able to do so, they can explore new dimension in induce mutagenesis. Study also encompasses characterisation of Cu- and CdS-NPs (using UV—visible spectra — UV—vis, Fourier transform infra-red spectroscopy — FTIR, X-ray diffraction — XRD, dynamic light scattering — DLS, field emission scanning electron microscopy — FESEM and transmission electron microscopy — TEM) for assessment of their nature and size. Furthermore, uptake of NPs is studied in seedlings by atomic absorption spectroscopy (AAS).

2. Materials and methods

2.1. Germplasm

Seed samples (moisture content: 5.0%; seed size: length — 2.85 mm ± 0.11, breadth — 1.68 mm ± 0.03; seed colour: jet black — RAL 9005-010-010) of *N. sativa* L. (Family: Ranunculaceae) was obtained from medicinal plant garden, Narendrapur Ramkrishna Mission, Government of West Bengal, India.

2.2. Preparation of copper and cadmium sulphide nanoparticles

Cu- and CdS-NPs were prepared using wet chemical co-precipitation methods as adopted earlier by Chatterjee et al. \cite{51} and Halder et al., \cite{11} respectively. Cu-NPs was prepared using 8 ml (10%, w/v) gelatin (Merck, India), 0.5 ml of 0.1 M CuCl\(_2\) (SRL, India) and 1.5 ml Milli-Q water (0.22 μ, Millpore membrane), and the ingredients were thoroughly mixed. Concentrated ammonia (25%, Merck, India; 3 drops) and hydrazine hydrate (5 M; 10 drops) were added drop wise into the solution, stirred for 3 minutes and left undisturbed until the characteristic reddish brown colouration was developed. The suspension was filtered (Whatman® No. 42) and stored in an airtight capped container. 

CdS-NPs were synthesised from drop-wise addition of freshly prepared Na\(_2\)S (16 ml) solution into the bi-compound mixture (18:16) of cadmium acetate and sodium dodecyl sulphate (SDS) under continuous stirring.

Bulk Cu and CdS (0.25 μg/ml) were prepared from the source compound used in NPs preparations without the capping agents (Cu: gelatin; CdS: SDS).

2.3. Assessment of nanoparticles

Opto-physical study of synthesised Cu- and CdS-NPs was carried out using UV—vis, FTIR — only for CdS-NPs, XRD, DLS, FESEM and TEM.
2.3.1. UV–vis spectrophotometry

UV–vis absorption spectra of Cu-NPs suspension were recorded in the wavelength ranging between 400 and 600 nm in Shimadzu UVPC-1601 UV–vis spectrophotometer, in diffused reflection mode. Gelatin solution containing ammonia and hydrazine hydrate (preparation identical to Cu-NPs suspension) was used as reference at room temperature (22 ± 1 °C).

Ethanol mounted CdS-NPs were analysed using ethanol as blank. Absorption of visible spectrum was studied between 200 and 600 nm.

2.3.2. FTIR

The powdered CdS-NPs were diluted (1:100) in potassium bromide (IR grade) for assessment of diffused reflectance at infra-red spectrum between the range of 400 and 4000 cm⁻¹ using FTIR analyser (Jasco FT/IR-6300 Fourier transform infrared spectrometer).

2.3.3. XRD

XRD patterns of powdered Cu- and CdS-NPs were calculated on Shimdzu LabX X-ray diffractometer using CuKα radiation (λ = 1.5406 Å) with 2θ ranging between 10° and 90° in steps of 0.02° and 1s per step sampling time. Crystallite size (Dnkl) was estimated using Debye–Scherrer equation, 

\[ D_{nkl} = \frac{k\lambda}{\beta \cos \theta} \]

where \( \lambda \) = wavelength of CuKα radiation, \( \beta \) = full width of half maximum intensity, \( \theta \) = diffraction angle in radian at diffraction peak and \( k \) = shape factor constant.

2.3.4. DLS

Average hydrodynamic diameters of both NPs were measured using DLS analyser (Delsa™ Nano C, Beckman Coulter).

2.3.5. FESEM and TEM

Particle surface ornamentation and surface morphology were studied using FESEM (JEOL JSM-7600F, resolution 1.0–1.5 nm) and TEM (JEOL JEM-2100HR, resolution 0.14–0.23 nm). For TEM study, 100 times diluted samples were dropped dried on a carbon-coated Cu grid (Applied Biosystems, India).

Bulk Cu and CdS were oven dried (≤60 °C for 4 h), and the obtained powdered samples were analysed under stereo microscope (Stereo Zoom® Leica S8 APO) for size determination.

2.4. Treatments

Dry seeds of *N. sativa* were exposed to Cu- and CdS-NPs (0.25, 0.50 and 1.0 μg/ml, 3 and 6 h durations), EMS (0.25, 0.50 and 1.0%, 3 and 6 h durations; solution prepared in 0.2 M phosphate buffer, 0.2 M KH₂PO₄ 49.7ml: 0.2 M K₂HPO₄ 50.3 ml, pH 6.8) and gamma irradiations (25, 50, 100, 200 and 300 Gy, source ⁶⁰Co, absorbance dose rate 47.4 Gy/min, source to distance 12 cm). Dry control and bulk Cu- and CdS controls (0.25 μg/ml, 3 h) were also kept for assessment. In each lot of treatments, 100 seeds were exposed.

2.5. Accumulation of Cu- and CdS-NPs in seedlings

A weight of 0.45 g ash (10 days old seedlings, dried at 300 °C for 24 h) for each treatment, including bulk controls of Cu and CdS, was dissolved in 25 ml concentrated tri-acid (nitric acid:sulphuric acid:perchloric acid::3:3:1) solution and the volume was reduced to 5 ml by heating under a fume hood (80 °C). The concentrated solutions were cooled to room temperature (24 ± 1 °C). Finally, the solutions were diluted by using ddH₂O up to 25 ml and filtered in a Whatman® filter paper (No. 42). The clear solutions (three replicas for each set) were analysed in a spectra AAS 240 (Agilent Technologies with 520 and 326.1 nm for Cu²⁺ and Cd²⁺ ions, respectively).
2.6. Assessment of seed germination and mitotic study

Fifty seeds from each lot of treatment along with controls (dry control and Cu and CdS control) were allowed to germinate in Petri plates lined with moist filter paper (22 ± 1 °C). Bursting of seed coat and emergence of radical were taken as an index of germination.

Six roots (about 2 mm length) from each control and treatments were cut, fixed in 1:3 acetic alcohol for overnight and preserved in 70% alcohol under refrigeration (16 ± 1 °C). For each treatment including control, three slides (replicas; randomly two roots were taken) were prepared from root tip squashes in 45% acetic acid and observed under Olympus trinocular light microscope (CH20i).

Mitotic index \[ (Mi) = \left( \frac{\text{total number of dividing cells}}{\text{total cells}} \right) \times 100 \], type of abnormalities in both dividing and resting cells and percentage of total aberrations in dividing cells were scored. Dose-wise representation of mitotic index and total frequency of abnormal dividing cells were graphically plotted for each treated materials (Cu- and CdS-NPs, EMS and gamma irradiations). Pooled data across the doses were tabulated. Photomicrographs were taken from suitable preparations.

2.7. Raising of plant population

Fifty seeds from each dose of treated materials including controls were sown in the experimental field plot of Department of Botany, University of Kalyani (West Bengal plain, Nadia, latitude 20°50’ to 24°11’ N, longitude 88°09’ to 88°09’ E, elevation 48 ft above sea level; sandy-loamy soil, organic carbon 0.76%, soil pH 6.85) in late November to raise the M1 plant population (15 cm between plants, 25 cm between rows). No fertiliser was applied during any stage of plant growth. The plants were harvested in mid-April.

2.8. Meiosis

For meiotic studies, floral buds of suitable sizes were fixed (5 a.m. to 6.30 a.m.) from individual plant of each treated dose (all surviving plants were scored) in acetic alcohol 1:3 for overnight and preserved in 70% alcohol under refrigeration. Pollen mother cells (PMCs) and pollen grains obtained from anther squash preparations were stained in 2% acetocarmine solution. Fully stained pollen grains were considered as fertile.[52] Data were scored from scattered metaphase I (MI) and anaphase I (AI) meiocytes and also from anaphase II (AII) cells. Pooled data across the doses were tabulated. Photomicrographs were taken from suitable preparations.

Frequency of seedless plants was counted on harvest from treated doses and controls.

3. Results

3.1. Characterisation of nanoparticles

Optical absorbance of NP suspension exhibits characteristic plasmon resonance peak at 574 nm for Cu-NPs (Figure 1(a)) and well-defined doublet exciton peak at 478 and 483 nm for CdS-NPs (Figure 1(b)).

Multiple inversed absorption peak bands for CdS-NPs, using FTIR analyser, are recorded in the spectrum regions of 400 to 420, 580 to 750, 1000 to 1050, 2260 to 2390 and 3000 to 3500 cm\(^{-1}\) (Figure 1(c)).

XRD pattern of powdered Cu-NPs shows characteristic sharp triplet peak at 43.3°, 51.5° and 74.6° (Figure 1(d)) corresponding to the index of scattering form (1, 1, 1), (2, 0, 0) and (2, 0, 0) crystal faces, respectively. Prominent broad diffraction peaks of CdS-NPs are observed at 26.6°, 44.2° and 53.02° (Figure 1(e)) and are considered as conformation of existence of (1, 1, 1), (2, 0, 0) and (3, 1, 1) planes, respectively. Average crystallite sizes of Cu- and CdS-NPs calculated from Scherrer equation are 43.08 nm ± 7.46 and 8.82 nm ± 0.91, respectively.
Hydrodynamic diameters of prepared Cu- and CdS-NPs analysed from diffused light scattering by particle Brownian motion are estimated to range from 25.7 to 120.4 nm (33.2 nm ± 9.6) (Figure 1(f)) and 29.4 to 115.7 nm (37.8 nm ± 10.7) (Figure 1(g)), respectively. Polydispersity indices are determined as 0.442 and 0.330 for Cu- and CdS-NPs, respectively.

Size of Cu- and CdS nanoclusters are determined from FESEM study and found to range from 220 to 800 nm (Figure 2(a)) and 20 to 800 nm (Figure 2(b)), respectively, with uneven surface topology.

Figure 1. (a–g) Characterisation of copper and cadmium sulphide nanoparticles. (a–b) UV–vis spectra of (a) Cu-NPs and (b) CdS-NPs. (c) FTIR spectra of CdS-NPs. (d–e) XRD patterns of powdered NPs ((d) Cu; (e) CdS). (f–g) DLS pattern of size distribution of (e) Cu-NPs and (f) CdS-NPs.

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TEM image of Cu-NPs reveals parabolic to hyperbolic crystals ranging from 24 to 117 nm (41.7 nm ± 9.4) centred with 12 to 38 nm (26.2 nm ± 8.7) metallic core (Figure 2(c)). The CdS NPs are spherical to cuboid in nature (Figure 2(d)) with 30 to 80 nm size range (37.4 nm ± 8.5).

Bulk Cu (range: 2.47 to 80.0 µm; mean: 14.87 µm ± 8.7) and CdS (range: 1.77 to 50.0 µm; mean: 10.11 µm ± 5.9) clusters are much higher in sizes than the prepared NPs.

### 3.2. Accumulation of Cu- and CdS-NPs detected in the form of Cu$^{2+}$ and Cd$^{2+}$

Cu- and CdS-NPs accumulation (µg/g) in seedlings is mostly dose-dependent (Table 1). Cu$^{2+}$ and Cd$^{2+}$ accumulation are also observed in low amount in bulk materials.

### 3.3. Petri plate germination frequency

As compared to controls (dry: 72%; bulk Cu control − 74%; bulk CdS control − 68%), Petri plate germination frequency is reduced mostly in all the doses of gamma irradiations (74% in 25 Gy to 12% in 300 Gy), EMS (66% in 0.25%, 6 h to 4% in 1.0%, 6 h), Cu-NPs (70% in 0.50 µg/ml, 6 h to 62% in 1.0 µg/ml, 3 h) and CdS-NPs (74% in 0.50 µg/ml, 6 h to 56% in 0.50 µg/ml, 3 h). Reduction in germination frequency is not dose-dependent and less affected in NP treatments than that of EMS and gamma irradiation.

### 3.4. Mitotic index and cytological aberration types and frequency

Mitotic index is 22% in both dry control and bulk Cu control and 18% in bulk CdS control. Compared to controls, significant ($p < 0.05$) reduction in the frequency of dividing cells is studied.
between/among doses (EMS: 11% to 5%, gamma irradiations: 18% to 8%, Cu-NPs: 11% to 6% and CdS-NPs: 22% to 7%) of treatments (Figure 3(a)–3(g)). Reduction in mitotic index is not dose-dependent. The effect is pronounced (excepting CdS-NPs, 6 h duration) in higher concentrations and prolonged duration (6 h) of treatments.

Mitotic cells at metaphase of untreated control show $2n = 12$ chromosomes always (Figure 4(a)). Bulk Cu- (3%) and CdS (2%) treatments show low frequency of metaphase cells with sticky and clumped configuration of chromosomes. Cytological aberrations studied in different treatments are diplochromatid nature (Figure 4(c)), stickiness, ring configuration (Figure 4(b)), fragmentation (Figure 4(b), 4(d), 4(e)), polyplid cell formation (Figure 4(d)), differential condensation (Figure 4(e)), bridges with or without fragment(s) (Figure 4(g) and 4(h)), laggards (Figure 4(f)), micronuclei (Figure 4(k)) and giant cell (Figure 4(l)). Chromatin fragmentation (Figure 4(j)), depletion of chromatin materials (Figure 4(i)) and cell and nuclear shape deformities are also observed. Bridges are mostly single and paired. CdS-NPs (0.50 $\mu$g/ml, 6 h) treatment shows criss-cross bridge formation. Bridges with fragment(s) are noted in 200 Gy gamma irradiations (9%) and in 1.0 $\mu$g/ml, 3 h CdS-NPs (1%) treatments. Micronuclei (1–3) of variable sizes are observed in all treatments. Micronuclei are either condensed or uncondensed type.

Total frequency of mitotic abnormalities is not dose-dependent and varied significantly ($p < 0.05$) between/among treatments (EMS: 9% to 39%, gamma irradiations: 13% to 57%, Cu-NPs: 13% to 26%, and CdS-NPs: 1% to 7%). Frequency of resting cells with micronuclei ranges from 1% to 2% in EMS, 1% to 17% in gamma irradiations, 0.5% to 3% in Cu-NPs and 1% to 7% in CdS-NPs. Polyploid cells with differential chromosome condensation (1%) are observed only in Cu-NPs treatments (0.50 and 1.0 $\mu$g/ml, 3 h; 0.25 $\mu$g/ml, 6 h).

Pooled data estimated across doses (Table 2) in treatments in comparison to controls reveal significant ($p < 0.05$) variation for mitotic index, frequency of abnormal dividing cells, fragments, bridges and micronuclei. Across treated materials, the frequency of abnormal dividing cells and micronuclei are in the following order: gamma irradiations > EMS > Cu-NPs > CdS-NPs and gamma irradiations > CdS-NPs > EMS > Cu-NPs, respectively.

### 3.5. Field germination frequency and survivability

As compared to controls (dry – 40%; bulk Cu – 36%; bulk CdS – 42%), germination frequency is reduced mostly in treatments (EMS: 18% to 14%, gamma irradiations: 22% to 6%, Cu-NPs: 36% to

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**Table 1. Accumulation Cu- and CdS-NPs in controls and treatments.**

| Treatments   | Dose (µg/ml) | Duration (h) | Amount of copper (Cu$_{2+}$/cadmium (Cd$_{2+}$) ions detected in seedling ash (µg/g) |
|--------------|--------------|--------------|--------------------------------------------------------------------------------|
| Bulk Cu      | 0.25         | 3            | 0.36                                                                               |
|              | 0.25         | 3            | 3.02                                                                               |
|              | 0.50         | 3            | 8.62                                                                               |
|              | 1.00         | 3            | 10.12                                                                              |
| Cu-NPs       | 0.25         | 6            | 4.94                                                                               |
|              | 0.50         | 6            | 9.80                                                                               |
|              | 1.00         | 6            | 10.76                                                                              |
| Bulk CdS     | 0.25         | 3            | 0.43                                                                               |
|              | 0.25         | 3            | 6.44                                                                               |
|              | 0.50         | 3            | 9.84                                                                               |
| CdS-NPs      | 1.00         | 3            | 13.49                                                                              |
|              | 0.25         | 6            | 8.37                                                                               |
|              | 0.50         | 6            | 9.07                                                                               |
|              | 1.00         | 6            | 19.11                                                                              |
Figure 3. (a–g) Mitotic index and frequency of abnormal dividing cells in relation to respective controls of *N. sativa*. (a–b) Cu-NPs. (c–d) CdS-NPs. (e–f) EMS. (g) Gamma irradiations. (A) Frequency of abnormal cells; (B) mitotic index.
Figure 4. (a–h) Mitotic cells ((a–e) metaphase, (f–h) anaphase) of *N. sativa* in (a) control and (b–i) Cu- and CdS-NPs treated material. (a) $2n = 12$. (b) Differentially condensed chromosome with a fragment (right-handed arrow) and a ring (dotted arrow). (c) Diplochromatid nature of chromosomes with unoriented chromosomes. (d) Cell with $>2n = 12$ chromosomes. (e) Differential condensation and fragmentation of chromosomes. (f) Anaphase with lagging fragments. (g) Chromosome bridge formation. (h) I: Anaphasic bridge with two equal size fragment, II: Double bridge with two minute fragments. (i) Cells without or with depleted chromatin. (j) Cell with fragmented chromatin mass. (k) Interphase cell with two unequal sized micronuclei. (l) Giant cell. Scale bar = 20 μm.
28%; CdS-NPs: 48% to 26%). Germination frequency is less affected in NP treatments than EMS and gamma irradiations. No germination of seeds could be recorded in 1.0%, 3 and 6 h EMS and 300 Gy gamma irradiations. Survivability of M₁ plants is 33% in EMS, 67% to 100% in gamma irradiations, 46% to 100% in Cu-NPs and 40% to 100% in CdS-NPs.

### 3.6. Meiotic studies

Pooled meiotic configurations across doses of treatments at MI, AI and AII and pollen grain fertility are presented in Table 3. PMC from control and treated plants mostly show 2n = 12 chromosomes (Figure 5(a)). Few meiocytes in NP treated materials (0.50 mg/ml, 6 h CdS-NPs) show variation in chromosome number at MI (nD 7/C0 0.86%/C0 Figure 5(j); nD > 6/C0 0.29%/C0 Figure 5(g)) and AI (2nD 24/C0 0.46%/C0 Figure 5(s)). Compared to respective control (5.88II/C0 0.24I to 6II), mean chromosome association per cell at MI among treatments ranges between 5.64II/C0 0.49I and 5.96II/C0 0.07I. Bivalent and univalent frequency per cell assessed across controls and treatments are non-random (p < 0.001) in distribution as evinced from \( \chi^2 \) test of heterogeneity (DF 6). In controls (including bulk materials), AI (100.0%) and AII (100.0%) cells are cytologically balanced (6/6/C0 Figure 5(n)) compared to treatments (AI: 72% to 96%, AII: 76% to 95%). Pollen grain fertility is reduced in treatments than controls (dry control/C0 96%; bulk Cu/C0 96%; bulk CdS/C0 90%; treated materials/C0 59% to 76%).

Abnormalities recorded in meiotic cells of treatments are sticky chromosomes, chromosomal fragments (1 to many/C0 Figure 5(b), 5(d), 5(g), 5(l)), lack of pairing between homologues,  

### Table 2. Pooled data across doses of treated materials in root tip mitosis of *N. sativa*.

| Treatments                  | Total no. of cells | Mitotic index (%) | Fragments (%) | Rings (%) | Bridges (%) | Bridges and fragments (%) | Total no. of abnormal dividing cells (%) | Micronuclei (%) |
|-----------------------------|--------------------|-------------------|---------------|-----------|-------------|--------------------------|----------------------------------------|----------------|
| Control                     | 905 704            | 22.21             | 0.00          | 0.00      | 0.00        | 0.00                     | 0.00                                    | 0.00          |
| Bulk Cu control (µg/ml)     | 712 554            | 22.19             | 0.00          | 0.00      | 0.00        | 0.00                     | 3.16                                    | 0.00          |
| Bulk CdS control (µg/ml)    | 1213 996           | 17.89             | 0.00          | 0.00      | 0.00        | 0.00                     | 2.30                                    | 0.00          |
| EMS (%)                     | 6977 6397          | 8.31              | 6.38          | 0.00      | 1.90        | 0.00                     | 19.32                                   | 1.33          |
| Gamma irradiation (Gy)      | 8093 7228          | 10.69             | 14.34         | 0.35      | 6.59        | 2.43                     | 46.67                                   | 6.95          |
| Cu-NPs (µg/ml)              | 10,222 9338        | 8.65              | 5.88          | 0.11      | 0.45        | 1.40                     | 17.84                                   | 1.29          |
| CdS-NPs (µg/ml)             | 6072 5198          | 14.34             | 0.69          | 0.00      | 0.23        | 0.00                     | 3.01                                    | 3.19          |
| CD at 5%                    | 6072 5198          | 14.34             | 0.69          | 0.00      | 0.23        | 0.00                     | 3.01                                    | 3.19          |
| p-Value at 6 DF             | <0.001             | <0.001            | <0.001        | <0.001    | <0.001      | <0.001                   | <0.001                                  | <0.001        |

### Table 3. Meiotic configurations and pollen fertility in treated materials.

| Treatments                  | Total no. of MI cells scored | Mean chromosome/cell | Total no. of AI cells scored | Balanced AI cells (%) | Total no. of pollen grains | Pollen fertility (%) |
|-----------------------------|-----------------------------|----------------------|-------------------------------|-----------------------|----------------------------|----------------------|
| Control                     | 300                         | 6.00                 | 400                           | 100.00                | 2158                       | 95.80                |
| Bulk Cu control (µg/ml)     | 225                         | 6.00                 | 337                           | 100.00                | 712                        | 95.79                |
| Bulk CdS control (µg/ml)    | 556                         | 5.88                 | 612                           | 100.00                | 681                        | 89.87                |
| EMS (%)                     | 1560                        | 5.89                 | 1668                          | 71.95                 | 2754                       | 59.37                |
| Gamma irradiation (Gy)      | 2341                        | 5.64                 | 1150                          | 84.78                 | 16,290                     | 69.12                |
| Cu-NPs (µg/ml)              | 1990                        | 5.96                 | 3930                          | 77.35                 | 8768                       | 75.58                |
| CdS-NPs (µg/ml)             | 2450                        | 5.83                 | 1974                          | 95.94                 | 6784                       | 76.34                |
| \( \chi^2 \) test of heterogeneity | 24.08             | 889.99               | 24.08                         | 889.99                | 2742                       | 89.46                | 6784 | 76.34 |
| p-Value at 6 DF             | <0.001                      | <0.001               | <0.001                        | <0.001                | <0.001                     | <0.001               |
Figure 5. Meiosis in (a) control and (b–x) nanoparticles treated plants. (a) 6II. (b) Pachytene stage with two fragments. (c) Diakinesis cell showing breakage of an arm (dotted arrow) in a bivalent. (d) MI with 5II + 2I + 3 fragments (right-handed arrow). (e–f) Chromosomal grouping (4II + 2II) at MI. (g) Fragments in association to bivalent and univalent at MI possibly 2n = 12. (h) Intense chromosomal fragmentation at MI. (i) Cytomicitic behaviour involving two meiocytes. (j) Hyperploid PMC = 7II at MI. (k) Hypoploid cell with condensed type chromosomal fragments. (l) Agglutination of chromatin and fragments. (m) Differential condensation of chromosomes at MI. (n) 6/6 separation at AI. (o) 3/5/4 separation of chromosomes at AI. (p) 5/7 AI separation with interchromosomal connections. (q) 2n > 12 with sticky chromosomes at AI. (r) Dicentric chromatid bridge with an acentric fragment. (s) 2n = 24; AI showing triphilar organisation of chromosomes. (t) All with one disorganised pole. (u) All with bridge. (v) Unequal tripolarity. (w,x) All with multiple groups and fragments. Scale bar = 10 \mu m.
differential chromosome condensation (Figure 5(m)), intense chromosomal fragmentation (Figure 5(h)), aneuploid variation in chromosome number (Figure 5(j) and 5(k)) and chromosomal grouping at MI (Figure 5(e) and 5(f)); laggards (1-2), unequal separation of chromosomes (5/7 – Figure 5(p) and 3/5/4 – Figure 5(o), 4/8 and possibly >2n = 12 – Figure 5(q)) and bridges with (Figure 5(r)) or without a fragment at AI; tripolarity (Figure 5(v)), unequal multipolar groups (Figure 5(w) and 5(x)), disorganised groups of chromosomes (Figure 5(t)), laggards and bridges at AII (Figure 5(u)). EMS and NPs treatments predominantly show meiocytes with sticky chromosomes at MI. Chromosomal fragmentation and multipolarity are observed in gamma irradiation and in NPs treatments. Cytomixis (Figure 5(i)) involving two PMCs (100 Gy gamma irradiations and 0.50 μg/ml, 6 h CdS-NPs; discrete in both cases) is also observed. PMCs squashes performed in a plant from 0.25 μg/ml, 3 h Cu-NPs treatment show total lack of microsporogenous tissues. Two meiocytes at pachytene (Cu-NPs: 1.0 μg/ml, 3 h) are with unequal sized fragments (Figure 5(b)). A PMC at diakinesis is found with arm breakage (Figure 5(c)). Relatively, gamma irradiations and NP treatments induce higher frequency of aberrations than EMS in meiotic cells.

After harvesting of all mature M1 plants, the frequency of seedless plants are counted as 0% in controls, 40% to 50% in gamma irradiations, 67% in EMS, 28% to 36% in Cu-NPs and 38% to 91% in CdS-NPs (Figure 6(a) – 6(d)).

4. Discussion

4.1. Characterisation of synthesised nanoparticles

Visible absorption of Cu-NPs showing prominent plasmon resonance peak at 574 nm confirms the presence of metallic Cu nanocrystallite in the suspension.[53] Spectral analysis of CdS-NPs exhibiting double excitation peak along with higher absorption efficiency towards lower spectral series.
indicates blue-shifting of light absorption. Increase in absorption tendency of both NPs at high energy band spectrum signifies decreasing size of the NPs. Sizing effect noted for CdS-NPs is the consequence of photon energy generated electron-hole pairs confinement at conductance band of semiconductor NP.

Infra-red absorption of CdS-NPs indicates the chemical purity as well as compositional complexity of the synthesised NPs. Small and weak transmissional depression between 400 and 420 cm$^{-1}$ establishes Cd–S bonding nature of the NPs. Absorption band of 550 to 750 cm$^{-1}$ is attributed to the crystalline disulphide (S–S) bond implying nanocrystal aggregates of CdS-NPs. Inverse peaks localised in 1000 to 1050, 2260 to 2390 and 3000 to 3500 cm$^{-1}$ are distinguishing signal of ‘S–O’, ‘S–H’ bond and intermolecular H-bonds, respectively. This explains the presence of lattice water as well as intermolecular water strongly attached to the surface of the CdS-NPs.

XRD pattern of Cu-NPs is well agreed with cubic nature of the metallic NP. Broad diffraction peaks of CdS-NPs also correspond with cubic phase confinement associated with decreasing size of quantum crystals. Baseline evenness in XRD along with low polydisperity index in DLS for both NPs signifies the purity and dispersity of the nanosuspensions. Size variation between DLS and XRD for the studied NPs is possibly due to progressive agglomerisation of the nanocrystals in the suspension. XRD shows efficiency in measuring nanograins even from the agglomerates; while, DLS only measures clustered grains in the suspension.

TEM micrographs of both metallic and semiconductor NPs nearly corroborate with the size measurement data obtained from DLS analysis. However, FESEM images of Cu- and CdS-NPs are not coinciding with the results of TEM and DLS as SEM measures the nanoclusters rather than individual crystalline grain. Size variation in NPs has been reported in assessment between SEM image and XRD method.

4.2. Mitotic and meiotic analyses

The present findings reveal that EMS, gamma irradiations and NPs cause cytotoxicity in mitotic cells by significantly ($p < 0.001$) reducing the frequency of dividing cells and inducing mitotic aberrations in relation to controls. Cytotoxic effects are reported earlier by conventional mutagens and NPs. Nagaonkar et al. reported that Cu-NPs cause a gradual reduction in mitotic index with concomitant increase in mitotic abnormality in *Allium cepa*. D’Amato suggested that suppression of mitotic index was due to ‘prophase poisoning’. Sparrow et al. reported that mitotic inhibition was related to physiological disturbances.

Pooled mitotic data across doses of treated materials including controls reveal significant variations ($p < 0.05$) in mitotic index, total frequency of mitotic abnormalities and types of aberration. Assessment of mitotic data signifies that NPs can cause similar consequences as that of EMS and gamma irradiations. Cu-NPs show higher degree of cytotoxicity than CdS-NPs. Mitotic aberrations noted due to treatments can broadly be categorised as (1) stickiness of chromosomes caused due to depolymerisation of DNA, (2) chromosome breakages leading to formation of rings, bridges, fragments and micronuclei, (3) inhibition of centromeric division bringing about the formation of diplochromosomes and (4) discrepancies in spindle apparatus giving rise to polyploid cells and laggards. Frequency and spectrum of mitotic aberrations and the occurrence of micronuclei in resting cells are considered as important tools to assess sensitivity of plant species towards treating agents, and form an integral part of study on induced mutagenesis.

Similar types of meiotic abnormalities are studied in different treatments. NPs induce distinct chromosomal breakages and the effect is intense in few PMCs (Cu-NPs). Aneuploid variations in chromosome number are scored in a plant following NPs treatment. PMCs squash preparation of the same plant showed cytomixis and it is possibly the contributing factor behind the aneuploid variation noted. Pollen fertility is reduced in treatments as compared to controls which signifies possible hindrance exerted by the treated materials either during pollen formation or in developmental maturation process or in both.
Types of mitotic and meiotic aberrations induced by Cu- and CdS-NPs resemble that of the conventional mutagens like EMS and gamma irradiation. Present findings indicate that the studied NPs possess mutagenic potentiality. Several mitotic cycles lead to meiotic cell division. NPs inducing aberrations in meiotic cells are significant as persistence of such changes can bring about heritable alteration in the genotype. In the context, it is noteworthy to mention that present group of workers have already reported that Cu- and CdS-NPs can induce heritable phenotypic changes in *M. uniflorum* (Lam.) Verdc. [11,12]

Occurrence of seedless plants at M1 in all the treated materials and more so in Cu- and CdS-NPs is rather significant. Seed formation is a result of cumulative effects of many important processes like sporogenesis, pollination, fertilisation and embryogeny. Conventional mutagens as well as NPs have affected any or all the sequential processes of seed development reflecting the possibility of genetical changes in the formed seeds.

5. Conclusion

EMS [78—80] and gamma irradiations [81,82] are effective mutagens that interacts with DNA to bring about mutational changes. NPs are also reported to interact with DNA in different plant species.[32,83—85] The present investigation highlights the potentiality of Cu- and CdS-NPs as mutagenic agents, a pioneer report of its kind.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Compliance with ethical standards

The research does not include any animals including human beings. Informed consent in this regard is not applicable.

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