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The Genotoxic Effect of Coal Fly Ash of Thermal Power Plant on *Raphanus sativus L.* (Radish)

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ABSTRACT

The effect of coal fly ash treatment on the chromosomes of *Raphanus sativus L.* was investigated. The seeds of *Raphanus sativusL.* were placed in petri dishes in three replicates and allowed to germinate for five days in different concentration of coal fly ash solution. The root were treated with diluted, semi-diluted, and concentrated solution of fly ash, while the control group had distilled water. The total aberration were examined. The mitotic index was calculated. The mitotic index decreased as the concentration increased. The highest mitotic index value was diluted fly ash solution while the least was concentrated fly ash treatment. The results show the most frequent chromosomal abnormalities observed included: chromatid bridge, c-mitosis, and stickiness. Concentrated fly ash solution is much more genotoxic than semi-diluted fly ash solution, as it induced more aberrations having percentage abnormalities for the highest concentration tested.

Keywords : Coal Fly-Ash, Genotoxic, Cytogenetic, Mitotic Index, *Raphanus Sativus L.*

I. INTRODUCTION

Coal is the only natural resource and fossil fuel available in abundance in India. Consequently, it is used widely as a thermal energy source and also as fuel for thermal power plants producing electricity. In India, the combustion of coal products are produced each year is around 112 MT (metric ton), which is likely to exceed and reach up to 170 MT by 2012. In India, The concentration of various elements also varies according to particle size [1], [2], [3]. Various elements that constitute fly ash are Si, Ca, Mg, Na, K, Cd, Pb, Co, Cu, Fe, Mn, Mo, Ni, Zn, B, F, Ca, and Al. Coal fly ash, contains Silica, Aluminium oxides of Iron, Magnesium, Calcium, Chromium, Arsenic, Lead, Zinc, Nickel and other toxic elements [4], [5]. Some possible agronomic uses of coal fly ash as, a fertilizer [6], [7], a limiting material [8] and as a physical amendment [9] have been indicated. Lower amendment levels of coal fly ash caused enhancement of both growth and yield while adverse effects at high levels were observed [10]. Radish (Raphanus sativus L.) is a good accumulator of heavy metals and has been widely used as an indicator plant in metal pollution experiments [11].

II. MATERIALS AND METHODS

Root tip excision and slide preparation

Dry seeds of each plants were spread uniformly in petri dishes lined with filter paper. The petri dishes were divided into three replicates. Equal volumes of the different concentrations of fly ash solutions (Diluted, Semi diluted and concentrated), were respectively admistrated while the control group had distilled water. The seeds were allowed to germinate within the petri dishes and were treated with the different concentrations of the fly ash and distilled water, respectively at a temperature of 26 ˚C for five to six days. The time of cutting the roots was critical factor since the rate of nuclei division was not constant throughout the day, in the morning was the highest.

Growing root tips were brittle, translucent and gently tapering were selected from the three plants grown in the solutions of different concentration and from the control. The control as well as coal fly ash treated root tips were fixed in carnoy's Fluid 3:1 methanol- acetic acid. The staining with aceto orcine was found suitable for present study. Temporary squash preparation of the material was found for preliminary observations and the preparations were made permanent with DPX. Mitotic index and percentage of aberrant cells was scored per thousand cell.

Temporary squash preparation of the material was used for preliminary observation and the preparation were made permanent with DPX. Mitotic index was scored after screening thousand of cells from each group. Percentage of aberrant cells was scored per thousands cell. The size of the cell calculated by stage micrometer and ocular micrometer.

Calculation of mitotic index (in percentage) and percentage of aberrant cells

Mitotic index was calculated by observing the slides root tip control and treated plants at five to six days of growth. About 1000 cells were scored for mitotic index and mitotic abnormalities were observed from dividing cells.

The cell in the stage of prophase, metaphase, anaphase and telophase were counted. The mitotic index was obtained as followed.

Mitotic index (%) = No. of dividing cells/ Total no. of cells studies × 100
Percentage of aberrant cells = No. of aberrant cells/ No .of dividing cells × 100

III. RESULTS

Raphanus sativus has a 2n=18 number of chromosomes. The anaphase, late metaphase and telophase were normal (Plate 1 A-b, B and C-c, D). Sticky metaphase (Plate 1 A-a), metaphase with micronuclei, coiling of chromosomes (Plate 1 C-a,b) observed. At SDS of fly ash treatment showed coiling of chromosome material (Plate 2 A, E), C-mitosis (Plate 2 C), sticky metaphase (Plate 2 D, F). At CS of fly ash coiling of chromatin material (Plate 3 A), sticky metaphase (Plate 3 B, C), C-metaphase , anaphase (Plate 3 D,F). Nuclear degeneration which leads to apoptotic cell (Plate 3 E). Similar dose effect was observed chromosome bridge at metaphase and sticky metaphase with micronuclei (Plate 4 A,B), sticky metaphase and C-mitosis (Plate 4 C,D).

The mitotic index presented in (Table 1). It was (2.200 %) at control, (7.001 %) with DS of fly ash, (3.190 %) with SDS of fly ash and (2.605 %) with CS of fly ash observed. Cell size of Raphanus sativus L. presented in (Table 2). It was (0.00140 mm) at control with 100X, (0.01710 mm) at DS of fly ash with 100X, (0.02500 mm) at SDS of fly ash with 100X, (0.03210 mm) at CS of fly ash with 100X observed during study.

IV. DISCUSSION

Fly ash contains heavy metals, which cause genetic damage to plants. Root of onion bulbs grown in fly ash polluted soil showed mitotic and chromosomal anomalies viz. clumping, stickiness, leggards and chromatin bridge formations. Fragments and c-mitosis appeared but in lower number due to heavy metal inhibition of spindle fibers [13]. The mitotic index of radish (*Raphanus sativus*) grown on soil amended with 10 to 50% fly ash showed an increase while at higher concentration (50-100%) of fly ash , clastogenicity and clumping of chromosomes was observed during mitotic divisions [14]. In present study, at SDS of fly treated root showed sticky metaphase, chromosome bridge at metaphase, disturb metaphase with vagrant chromosome, laggard

chromosome at anaphase, degradation of chromatin material during study. Mitotic index also decreased with increased concentration of fly ash in *Arachis hypogaea.*

Fly ash amended soil was used as growing medium for diploid and tetraploid maize crop but no triploid plants was observed at any fly ash concentration [15]. Leaching of coal fly ash before admixing with soil reduced its genotoxic effect. Fly ash that had been weathered by leaching for one week did not cause changes in the mean DNA amount, and cell cycle [16]. In present investigations investigations it was revealed that higher dose of fly ash were mitodepressive and induced various types of chromosomal aberrations in root tip cells of all plants. After treatment with various concentration of fly ash in *Raphanus sativus* at SDS of fly ash showed coiling of chromosome material, C-mitosis, sticky metaphase, C-metaphase observed. Similarly nuclear degeneration which leads to apoptotic cell at CS o fly ash.

Metal induced chromosomal stickiness has been reported to interfere with cell division [17], [18], reported the interference of Pb with cell division in grass pea. Pb was also found to inhibit root growth and cell division in *Zea maizes L.* [19]. Metal induced chromosomal stickiness accomained by pyknosis and chromatin degradation has also been reported in maize by [20], *Allium cepa* [21] and *Helianthus annuus* [22], these metal include Pb and Zn. Chromosome stickiness which was observed can lead to sticky metaphase and precocious sepration of chromosomes. This may be due to breaking of the protein moiety of the nucleoprotein backbone by these heavy metals (Pb and Zn). [23] had similar observations with chemicals tested on *Alium cepa*. Scattering which involves chromosomes spreading irregularly over the cell may be due to disturbance of the spindle apparatus by the metals. Anaphasic bridges formed were probably due to unequal exchange or dicentric chromosomes.

V. CONCLUSION

In conclusion Mitotic index was frequently reduced due to the increase in fly ash concentration and percentage of aberrant cells was increased with the SDS, CS of fly ash treatment on root tip cells. Cell size also increased gradually at SDS and CS of fly ash application.

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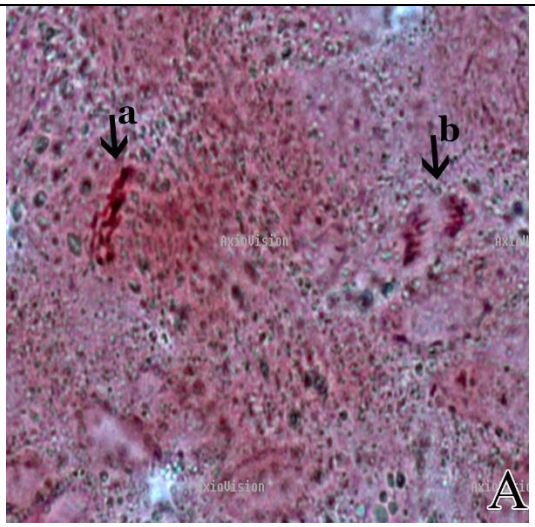
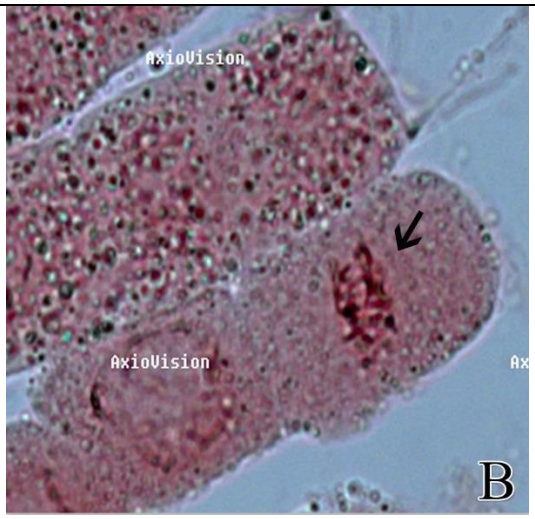
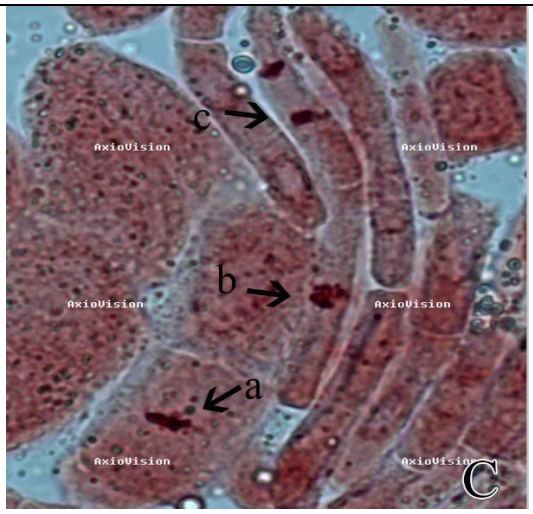


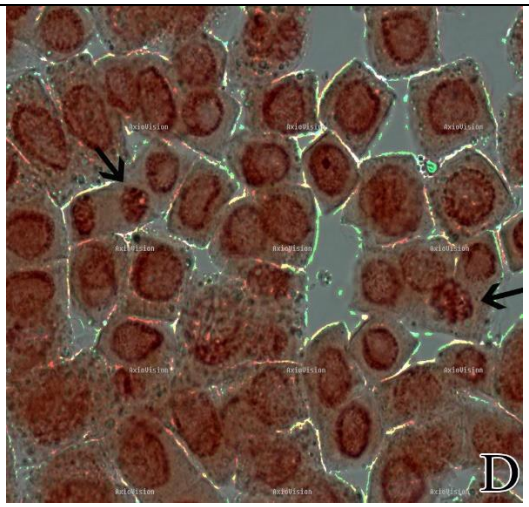
A. **a.** Sticky metaphase ; **b.** Normal anaphase



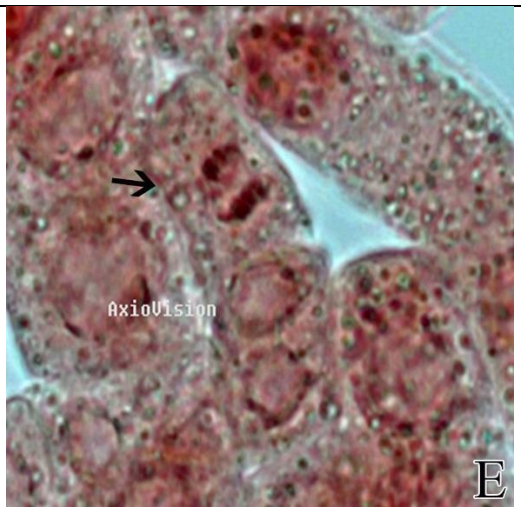
B. Normal late metaphase



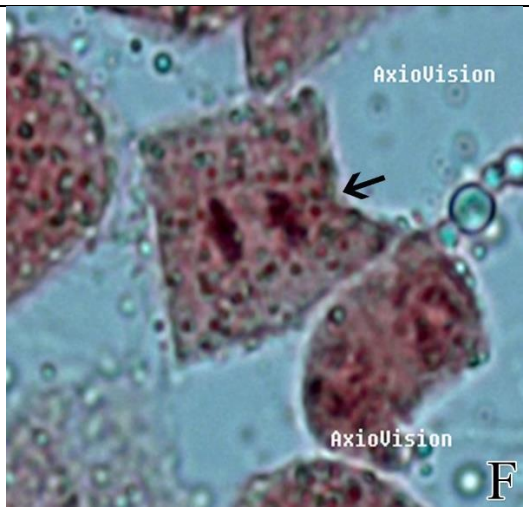
C. **a.** Metaphase with micronuclei; **b.** Coiling of chromosomes; **c.** Normal telophase



D. Telophase

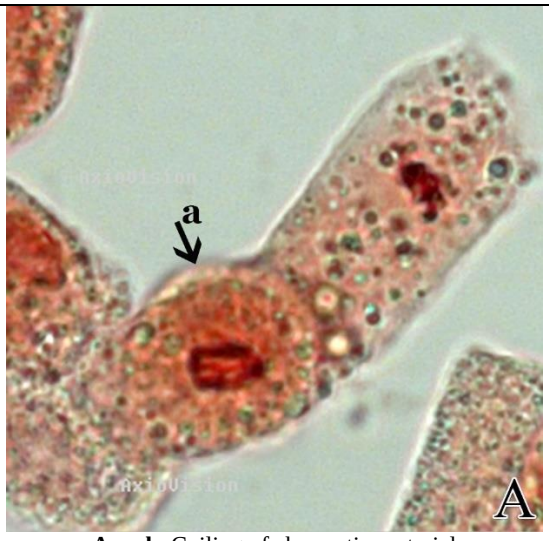


E. Anaphase

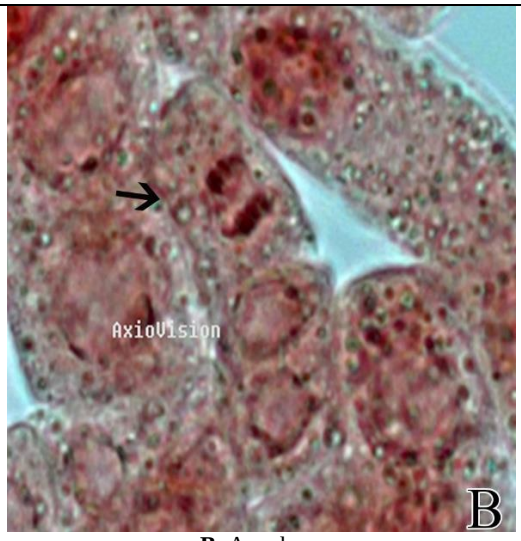


F. Anaphase

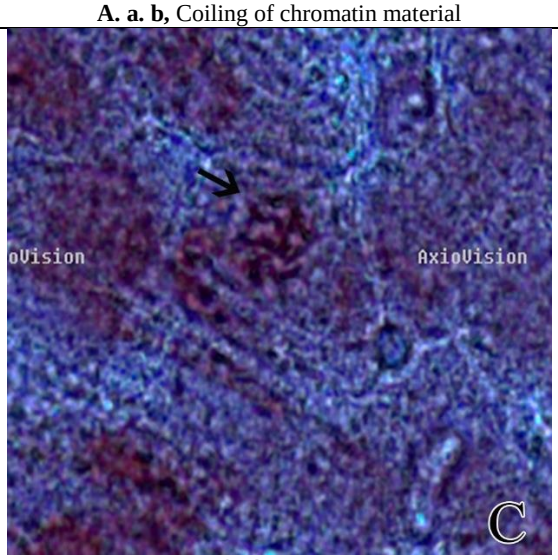
PLATE 1. A,B,D,E,F. Smear preparation of control roots of Raphanus sativus L
C. Smear preparation of fly ash treated roots of Raphanus sativus L



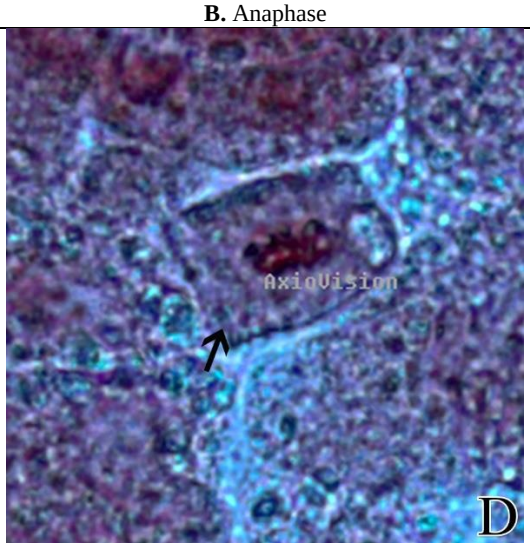
A. a. b, Coiling of chromatin material



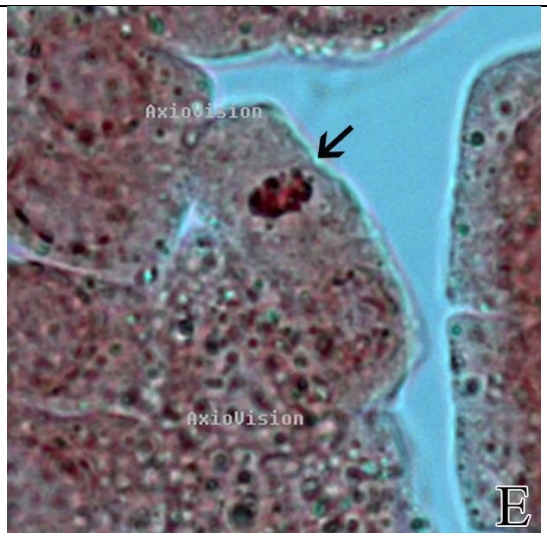
B. Anaphase



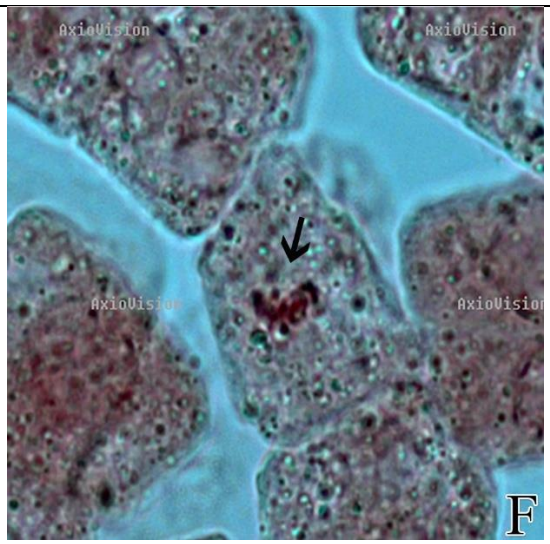
C. C-mitosis



D. Sticky metaphase

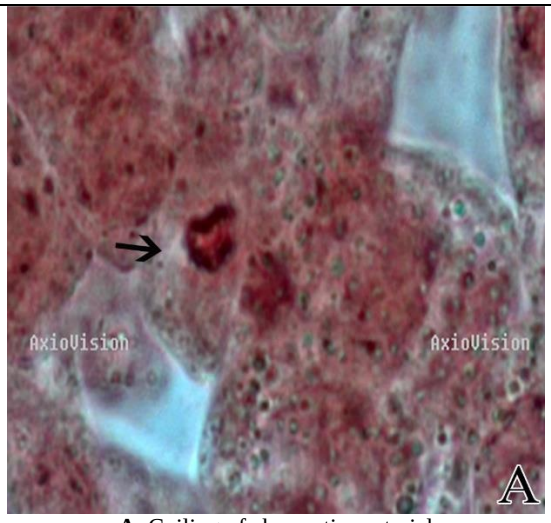


E. Coiling of chromatin material

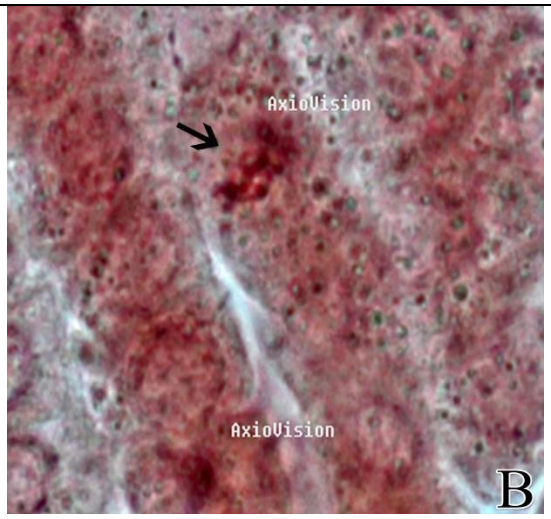


F. Sticky metaphase

PLATE 2. A-F. Smear preparation at fly ash treated roots of Raphanus sativus L



A. Coiling of chromatin material



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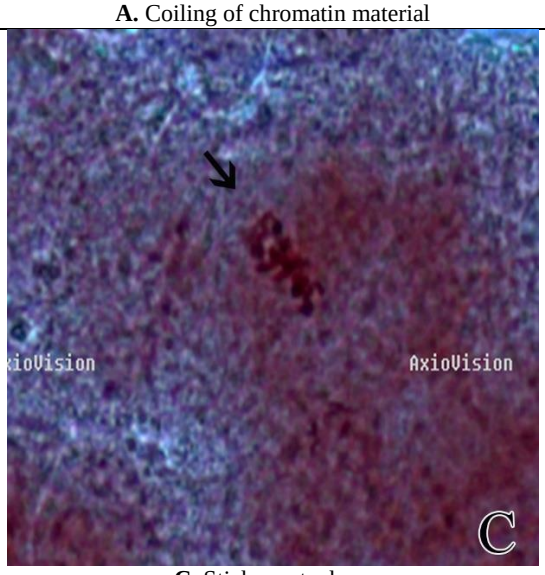
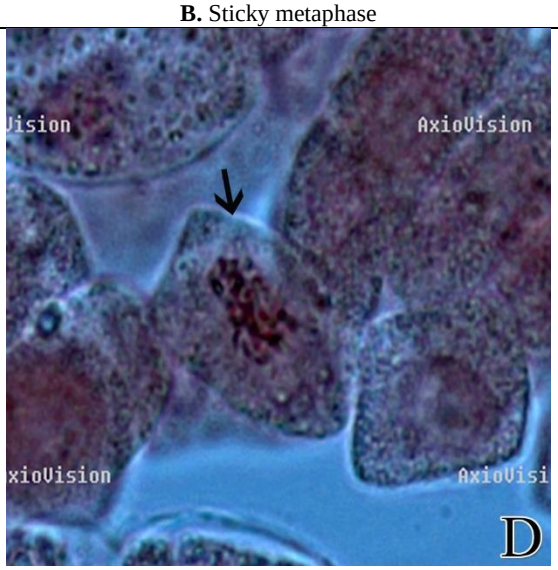
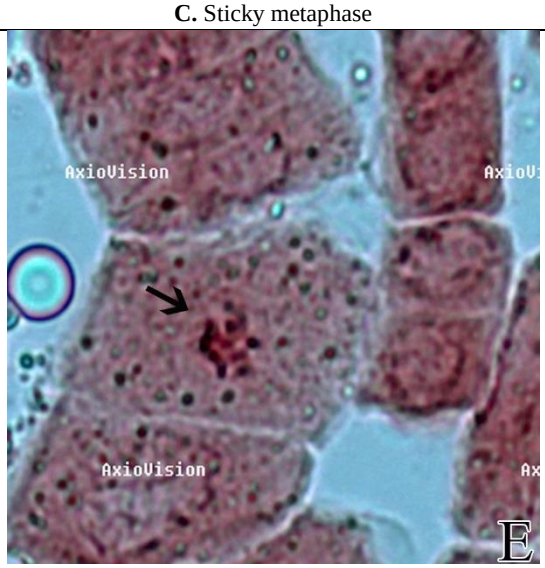


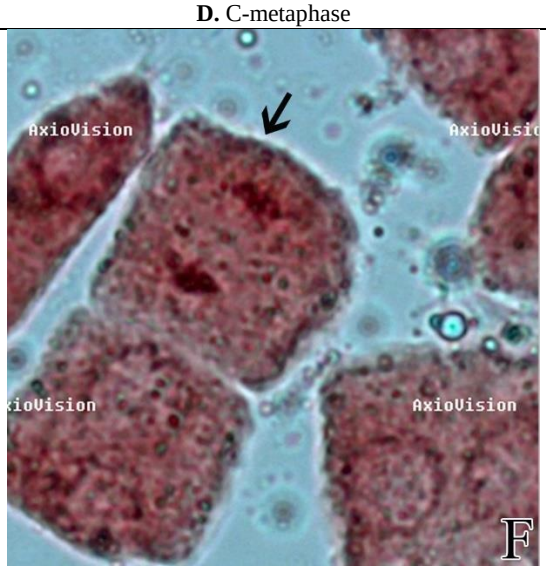
C. Sticky metaphase



D. C-metaphase

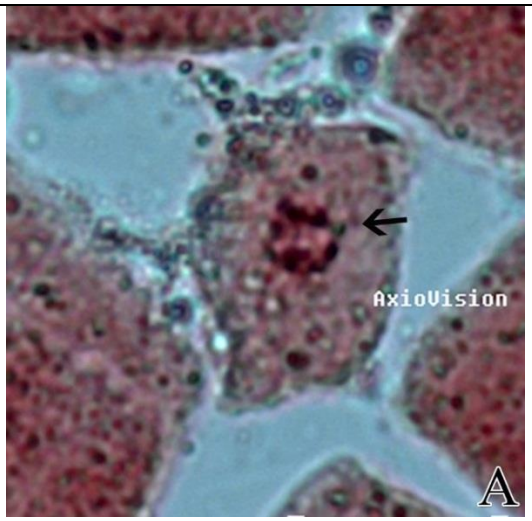


E. Nuclear degeneration

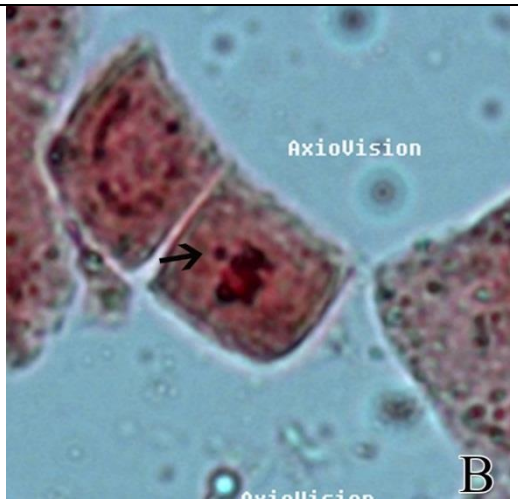


F. Anaphase

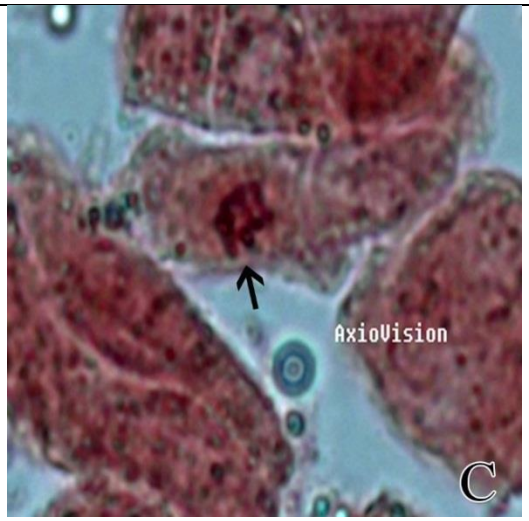
PLATE 3. A-F. Smear preparation at fly ash treated roots of Raphanus sativus L



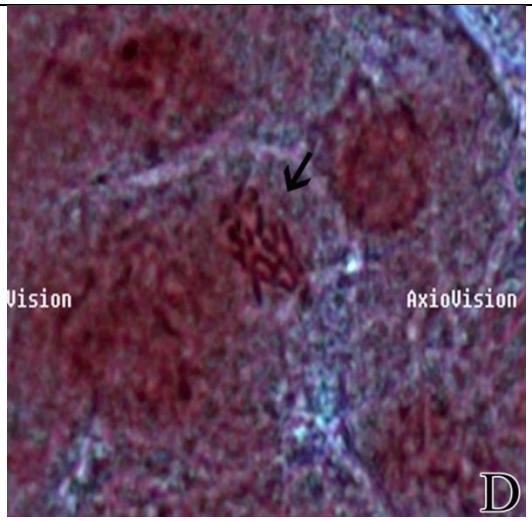
A. Chromosome bridge at metaphase



B. Sticky metaphase with micronuclei



C. Sticky metaphase



D. C-mitosis

PLATE 4. A-D. Smear preparation at fly ash treated roots of *Raphanus sativus L*

Table 1. Effect of Coal Fly Ash on root tip cell of Raphanus sativus L.

	Control	**DS**	**SDS**	**CS**
Mitotic index (%)	2.200 ± 0.020	7.001 ± 0.040	3.190 ± 0.118	2.605 ± 0.059
Aberrant cell (%)	4.450 ± 0.169	25.520 ± 1.040	50.046 ± 0.502	78.260 ± 0.645

Table 2. Effect of Coal Fly Ash on Cell Size of Raphanus sativus L.

	Control	**DS**	**SDS**	**CS**
10X	0.00170 ± 0.00012	0.01680 ± 0.00076	0.01600 ± 0.00010	0.03280 ± 0.00076
40X	0.00170 ± 0.00014	0.01800 ± 0.00010	0.02520 ± 0.00076	0.03300 ± 0.00010
100X	0.00140 ± 0.00012	0.01710 ± 0.00075	0.02500 ± 0.00074	0.03210 ± 0.00071