Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
(Print copies up to 2016)
e-ISSN: 2395-3454

Vol. 22 | No. 3 | pp. 1555-1562 | 2023

Original Research Paper

https://doi.org/10.46488/NEPT.2023.v22i03.041

Open Access Journal

Influence of Vermicomposted Coal Fly Ash on Morphological and Cytological Attributes of *Ricinus communis* L.

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Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 02-11-2022
Revised: 03-01-2023
Accepted: 19-01-2023

Key Words:
Fly ash
Pressmud
Vermicompost
Eisenia foetida
Ricinus communis L.

ABSTRACT

In view of the environmental problems generated by the large-scale production of fly ash, increasing attention is now being paid to the recycling of fly ash as a good source of nutrients. To reduce the cost of fly ash disposal and best utilization, it aimed to convert the fly ash into valuable vermicompost. Stated throughout the experiment, we opted for a soil sample and fly ash and pressed with different concentrations (control, 20%, 50%, 80% and 100%). Subsequently, all the mixtures were vermicomposted for 60 days by adding 100 Earthworms (*Eisenia foetida*) in each pile. The X-ray fluorescence spectroscopy measured the composition of the metal in fly ash as well as the nutritional content in the soil. This is followed by examining the morphological characteristics and cytogenetic study of *Ricinus communis* L. The present study indicated that *E. foetida* mitigates the toxicity of fly ash and is hence used as valuable vermicompost.

INTRODUCTION

To being with, due to this modernization came along with industrialization in metropolitan cities, electricity demand deliberately increased in day-to-day life (Mistry & Jadav 2018) (Raval et al. 2018) whereas in developing countries, depending upon the major source of fuel as coal; despite coal near to soil surface makes it easily minable and thus is relatively less expensive than hydro or nuclear power-based generations of electricity. In India, major power plants are using coal as fuel though alternatives have been searched for more than the last 10 years. Still, no feasible replacement for coal is available in India (Sharma & Akhai 2019). Lignite, bituminous, and anthracite are classified into organic maturity coal, which (proportion may differ) contains magnesium, calcium, and sulfate. Coal-based thermal plants contribute to major electricity production in India. From all these thermal power plants, dry fly ash has been collected through Electrostatic Precipitator (ESP) in dry conditions, and pond ash from ash ponds in semi-wet conditions (Ahmad 2015). Fly ash composes of fine glass, which has a spherical shape and ranges from approximately ~0.5 to 100μm. There has been seen that mainly two types were found (Mupambwa et al. 2015). Fly ash is used in the industry for manufacturing cement. Around 2.45 million tons of fly ash were used in 1998-1999. Whereas the disposal of fly ash is either dry state or wet state, even though the process of leaching the heavy metals, Wet state disposal facilitates in biomagnification of toxic components. The coal ash by-product has been classified as a Green List waste under the Organization for Economic Cooperation and Development (OECD) (Khan et al. 2013).

Fly Ash contains nutrients such as S, B, Ca, Mg, Fe, Cu, Zn, Mn, and P that benefit plants. It also contains toxic metals, including Cr, Pb, Hg, Ni, V, As, and Ba. Adding Fly Ash increases the availability of Na, K, Ca, Mg, B, and other nutrients except for N (Sharma & Kalra 2006). Thus, it was found that this material could be used as an additive/amendment material in agriculture applications. Some experience was gained in the country and abroad regarding the effect of fly ash utilization in agriculture & related applications (Swamy et al. 2010). In addition, Fly Ash significantly influences soil physical properties

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such as water-holding capacity and aggregation (Daniels et al. 2002).

MATERIALS AND METHODS

Sample Collection of Fly Ash

Fly Ash was collected in a clean bag directly from the Electrostatic Precipitator (ESP) of the Thermal Power Plant, Ukai Dam, on the Tapi River in the Tapi district of Gujarat, India.

Soil Sample Collection

The soil sample was collected from an agricultural field in the Bardoli region of Surat, Gujarat, India. Plants were grown in the same soil sample throughout the research period.

Pressmud Collection

Pressmud is a solid waste generated from Sugarcane in Sugar factories. Pressmud is a rich source of organic compounds. It was collected from Shree Khedut Sahakari Khand Udhyog Mandali Ltd., Sugar Factory, Madhi, Surat, Gujarat, India.

Seeds Collection

Seeds of *Ricinus communis* L. were purchased from Agriculture Produce Market Committee, Surat. Seeds were surface sterilized with H_2O_2.

Analysis of Fly Ash

X-Ray Fluorescence Spectroscopy detected metal in Fly Ash samples at SVNIT, Surat. Fe, Zn, Cu, Mn, and S were checked at Soil Testing Laboratory, Bardoli. Other physicochemical parameters such as pH, Electrical Conductivity, Organic Carbon (%), Available Potash (%), and Available Phosphate (%) were also checked at Soil Testing Laboratory, Bardoli.

Analysis of Soil

X-Ray Fluorescence Spectroscopy did Metal Detection in Soil samples at SVNIT, Surat. Virgin Soil (Control Soil) and different vermicomposted mixtures of Fly Ash, Pressmud, and Soil were tested for the same. Ca, Mg, and Na were checked in the control soil at Pollucon Laboratories, Surat. Other physicochemical parameters such as pH, Electrical Conductivity, Organic Carbon (%), Available Potash (%), and Available Phosphate (%) were also checked in Control Soil at Soil Testing Laboratory, Bardoli.

Vermicomposting of Mixtures

Different mixtures of Fly Ash, Pressmud, and Soil were prepared in different concentrations (Control, 20%, 50%, 80%, and 100%). All the mixtures were vermicomposted for 60 days by adding 100 Earthworms *(Eisenia foetida)* in each pile. The effect of Fly Ash on Earthworms was also checked in each concentration for 60 days at 15 days, in which numbers of live adult earthworms and numbers of Juveniles were counted. Ca, Mg, and Na were checked in these vermicomposted mixtures at Pollucon laboratories in Surat. Nitrogen, phosphorus, and potassium content were also checked in the vermicomposted mixtures at Soil Testing Laboratories, Bardoli.

Pot Experiment

Initially, the experiment was carried out with three controls, i.e., Control Soil, Soil and Vermicompost, and Soil and Pressmud. For the test, the mixtures of Soil, Vermicompost, Pressmud, and different concentrations of Fly Ash were taken, and 10 seeds were planted in each pot. The result was improved in the soil, vermicompost, pressmud, and fly ash mixtures. And so these mixtures with different concentrations of Fly Ash were further taken for the next set of experiments. The final experiment was carried out in black polyethylene bags and plastic pots varying in size. 5 kg of the prepared vermicomposted mixture was taken, and 10 seeds were added to each pot. Vermicomposted mixtures taken for the experiment were (Control, 20%, 50%, 80%, and 100%.). Tap water was used for the irrigation. The study was carried out in two phases. Initially, the plants were grown for 30 days and studied for 10 days. Later, plants were grown and studied up to maturation.

The parameters included in the plant study are as follows:

Seed Germination

Ricinus communis L. seeds were procured from Agricultural Produce Market Committee (APMC), Surat. The germination was carried out in pots. Seeds were surface sterilized with H_2O_2 to prevent surface fungal/bacterial contamination. Different concentrations of vermicomposted Fly Ash, viz. 20%, 50%, 80%, and 100%, were prepared, and tap water was used as a control for the study. Ten seeds were sown in the pots.

Cytogenetic Study

Slide preparation: Root tips were taken from seeds grown in different concentrations of Fly Ash, stained by the Darlington & La Cour (1976) method (Chakraborty et al. 2009). The root tips of different plants were collected at a particular time. The root tips were fixed in 3:1 Methanol: Acetic Acid for 12 hours, followed by hydrolysis in 5 N HC1 for 30 minutes. The root tips were washed 3 times with distilled water and stained in 1:1 Acetoorcein: Acetocarmine for 30 minutes.

Slides were observed under Carl-Zeiss Axioscope after the mitotic squash preparations. Various stages of mitosis, such as Prophase, Metaphase, Anaphase, and Telophase, were observed. Mitotic Index and Percentage Chromosomal Aberrations were counted. Mitotic squash preparations were made, and scoring was done to determine the mitotic index and the percentage of chromosomal aberration.

Calculation of the percentage of the mitotic index and percentage of aberrant cells: Mitotic index and aberrant cells were scored per hundred cells from the slides prepared from treated and control plants. Various stages of mitosis viz. prophase, metaphase, anaphase, and telophase were counted. The percentage of the mitotic index and percentage of aberrant cells was calculated using the following formulas.

$$\text{Mitotic index} = \frac{No.of\ dividing\ cells}{Total\ No.of\ cells\ studied} \times 100$$

$$\text{Aberrant cell} = \frac{No.of\ aberrant\ cells}{No.of\ dividing\ cells} \times 100$$

RESULTS AND DISCUSSIONS

Analysis of Soil

X-ray fluorescence spectrometry of fly ash: In the present study, X-Ray Fluorescence Spectrometry was performed for metal detection in Fly Ash. XRF was done at SVNIT, Surat. Table 1 illustrates the Fly Ash composition used throughout the experiment. Firstly, Silica was found to be the main content with 52.07%. Other than it, Iron and Calcium were present in higher amounts, 12.0853%, and 10.1632%, respectively. Secondly, Magnesium was present in moderate amounts, which was 3.7293%, Potassium was 3.1954% present, and 3.0246% Phosphorus was present in Fly Ash, while 3.41%Aluminium was also detected. Sulfur was 1.4832%, and Titanium was 1.7473% present in Fly Ash. Lastly,0.184% Nickel, 0.3215% Chlorine, 0.3178% Manganese, 0.4212% Rhodium, and 0.4778% Palladium were also detected in Fly Ash. At the same time, a very less amount of Copper (0.0371%), Strontium (0.0569%), and Zirconium (0.0536%) was detected in Fly Ash by X-Ray Fluorescence Spectrometry. The analysis of soil shows that the soil contains all the necessary elements required for healthy plant growth. The control soil sample was checked for the following parameters in Table 2, indicating that physical-chemical parameters of soil such as pH (5.76), EC millimhos/cm (0.40), Organic carbon (0.69), Available potash % (5.53), and Available phosphate % (0.418) were present in a requisite level of germination of seeds. Table 3 lucid that nutritional content was present in the soil, a mixture of fly ash, Pressmud, and Farm Soil. In this method, elements presented in soil are detected, namely calcium, magnesium, sodium, nitrogen, phosphorus, and potassium. Moreover, we opted for different concertation such as 20%, 50%, 80%, and control. 20% concertation was optimistic among the all. Around 0.13 % calcium, 0.031% magnesium, 3.71% nitrogen, and around 536 ppm sodium were present. In addition, the mixture contained 24 and 156 (kg.hectare^{-1}) of phosphorus and potassium. Mineralogically, fly ash is similar to the soil but rich in macro and micronutrients. The major attribute which makes fly ash suitable for agriculture is its texture and the fact that it contains almost all the essential plant nutrients except organic carbon and nitrogen (Kumar et al. 2005).

Germination

After plantation, the seed germination in control and 20% Fly Ash concentration was observed within 2 to 3 days in *Ricinus communis* L., while it was observed within 3 to 4 days in other concentrations. Overall, in the plant, seed germination was delayed with the increased Fly Ash concentration.

Table 1: XRF analysis of fly ash.

Elements	Amount present %
MgO	3.7293
Al_2O_3	3.41
SiO2	52.0781
P_2O_5	3.0246
SO3	1.4832
Cl	0.3215
K_2O	3.1954
CaO	10.1632
TiO_2	1.7473
MnO	0.3178
$Fe2O_3$	12.0853
CuO	0.0371
SrO	0.0569
ZrO_2	0.0536
Rh_2O_3	0.4212
PdO	0.4778
NiO	0.184
CaO	10.1632
TiO_2	1.7473

Table 2: Physicochemical properties of soil.

Parameters	Results
pH	5.76
EC [milimohs.cm^{-1}]	0.40
Organic Carbon %	0.69
Available Potash %	5.53
Available Phosphate %	0.418

Table 3: Soil Nutritional Content.

Elements	Control	20%	50%	80%
Ca (%)	0.04	0.13	0.13	0.22
Mg (%]	0.007	0.031	0.031	0.06
Na [ppm]	460	536	1115	1133
N [%]	3.62	3.71	3.89	2.85
P [kg.hectare^{-1}]	21	24	29	24
K [kg.hectare^{-1}]	130	156	134	132

Morphological Characteristics

The Shoot Length, Root Length, Number of Leaves, Fresh Weight, and Dry weight were noted on the germination's 10th (Fig. 1), 20th (Fig. 2), and 30th (Fig. 3) days. The parameters were noted as highest in control on the 10th day and highest at 20% on the 20th and 30th days. Shoot, Root Length, and Fresh Weight of Pods were also checked in mature plants (Fig. 4). All the parameters were noted the best in 20% of the Fly Ash concentrated mixture. A comparison was made between control soil and vermicomposted fly ash. Initially, the Morphology of the plant was observed. In 2005, Sharma and his co-worker (Jaroli & Sharma 2005) reported that since fly ash comprises useful nutrients, it enhances the availability of the micro and macro elements for plants. Observations noted after 10 days depicted that the root length initially increased when the concentration increased to 20% (Fig. 1). The length was 12.86 cm as opposed to 9.43cm for control. With the increase in concentration to 50 %, the length decreased to 10.36 cm (Fig. 3).

Further Increase in concentration ultimately resulted in a decrease in the root length. Thus, vermicomposted fly ash suitably favors root length growth at optimal concentration, but the effects are reversed with increased concentrations. Studies by (Emamverdian et al. 2015) have

Fig. 1: Growth of *Ricinus communis* L. on the 10th Day.

Fig. 2: Growth of *Ricinus communis* L. on the 20th Day.

Fig. 3: Growth of *Ricinus communis* L. on the 30th Day.

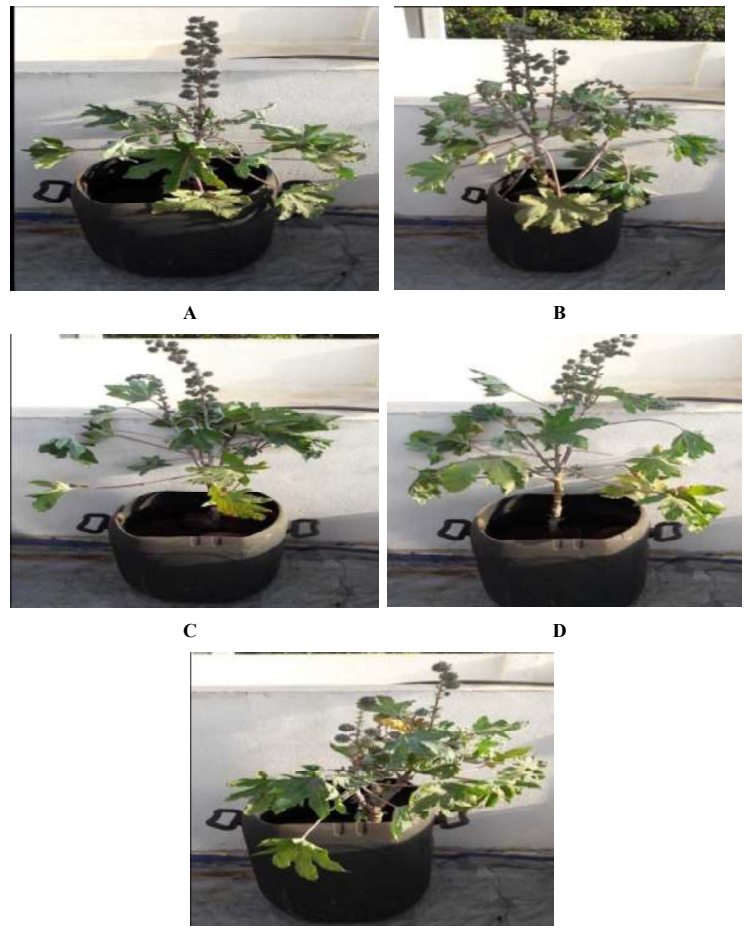
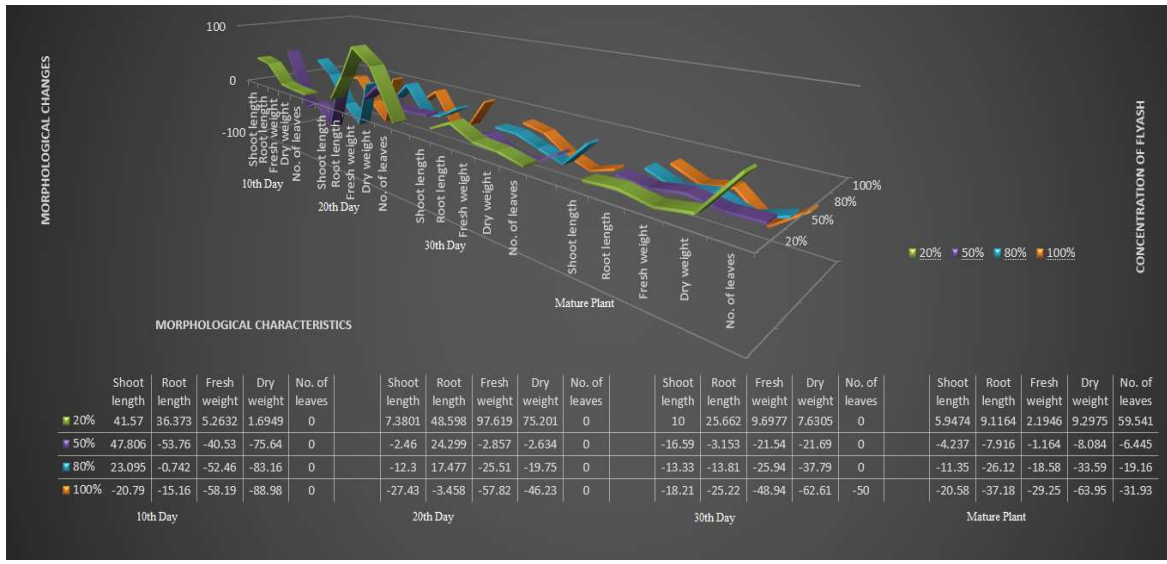


Fig. 4: Mature plants of *Ricinus communis* L. in different concentrations of Fly ash.
A. Plant is grown in Control.
B. Plant is grown in 20% fly ash.
C. Plant is grown in 50% fly ash.
D. Plant is grown in 80% fly ash.
E. Plant is grown in 100% fly ash.



	Shoot length	Root length	Fresh weight	Dry weight	No. of leaves	Shoot length	Root length	Fresh weight	Dry weight	No. of leaves	Shoot length	Root length	Fresh weight	Dry weight	No. of leaves	Shoot length	Root length	Fresh weight	Dry weight	No. of leaves
20%	41.57	36.373	5.2632	1.6949	0	7.3801	48.598	97.619	75.201	0	10	25.662	9.6977	7.6305	0	5.9474	9.1164	2.1946	9.2975	59.541
50%	47.806	-53.76	-40.53	-75.64	0	-2.46	24.299	-2.857	-2.634	0	-16.59	-3.153	-21.54	-21.69	0	-4.237	-7.916	-1.164	-8.084	-6.445
80%	23.095	-0.742	-52.46	-83.16	0	-12.3	17.477	-25.51	-19.75	0	-13.33	-13.81	-25.94	-37.79	0	-11.35	-26.12	-18.58	-33.59	-19.16
100%	-20.79	-15.16	-58.19	-88.98	0	-27.43	-3.458	-57.82	-46.23	0	-18.21	-25.22	-48.94	-62.61	-50	-20.58	-37.18	-29.25	-63.95	-31.93
	10th Day					20th Day					30th Day					Mature Plant				

Fig. 5: Graphical representation of morphological changes occurred in *Ricinus communis* L.

highlighted that fly ash benefits plants up to some optimum concentration.

The given histogram (Fig. 5) illustrates the morphological changes that occurred in *Ricinus communis* L. during the addition of fly ash with different concentrations of 20%, 50%, 80%, and 100%. The primary changes transpire in the shoot length, root length, number of leaves, measured fresh weight, and dry weight in different time intervals such as the 10th day, 20th day, 30th day, and after the plant's maturation. A cursory glance shows that the leaves emerged after the plant's maturation in a 20% fly ash concentration. Approximately 60 leaves were observed.

To start with, among all the concentrations of fly ash, 20% was optimized and gave a pleasant outcome. In the shoot length, the concentration of fly ash 20% and 50% give around 42% and 48%growth, respectively (all these percentages are compared with control), which was maximum on the 10^{th}-day incubation. Speculating more, after the 20^{th} and 30^{th} days of incubation, shoot lengths reach up to 8% and 10%, respectively, greater than the control. After the plant's maturation, around 6% of the shoot length was greater than the control plant. In contrast, oddly, 50%, 80%, and 100% concentrations of fly ash give a negative outcome (except on 10^{th}-day incubation in 80%), showing the inhibitory effect on plant growth, indicating that it would be catastrophic.

Moreover, in root length among all the incubation with fly ash on the 10^{th} day, 20% was decent for the growth around 37%, and if we talk about 50%, 80%, and 100% provide negative aftermath after the 20^{th}-day incubation gives better growth in 20%, 50% and 80% around 49%,24%, and 18% respectively, somehow in 100% obtained negative stimulation. On the 30^{th} day, 20% fly ash concentration gives 26%; after the plant's maturation, it gets 10% higher than the control plant. It also indicated that fly ash was also responsible for the development of roots.

With germination, plants gain some weight for their physical stability. Here we scaled two types of weights, first fresh weight, and second dry weight. It has been seen in the graph overall, only 20% could be the best. More precisely, on the 10^{th} day of incubation, fresh weight increased by around 5.3%. On the 20^{th} day, it reached up to 98%, and on the 30^{th} day, around 10% increment was noted, whereas at the maturation, around 2.1% weight was increased in the control plant. However, 50%, 80%, and 100% had given negative stimulation for the plant weight. This elucidation indicated that the huge concertation of fly ash inhibited plant growth. Besides, the same outcome was obtained on the 10^{th} day of incubation; somehow, dry weight in 20% fly ash concentration increased by 1.7%. On the 20^{th} day, it increased to around 75%. On the 30^{th} day, it was around 8%, and finally, at the maturation, approximately a 9.3% increment from the control plant was noted. Also, 50%, 80%, and 100% did not satisfy results, were observed. This indicated that higher fly ash concentration shows detrimental consequences for their stimulation.

Cytogenetic Study

The chromosome number in *Ricinus communis* L. is 2n=20. Cytological effects of vermicomposted fly ash with different concentrations were checked in *Ricinus communis* L. root

Table 4: Effect of different concentrations of vermicomposted fly ash on root tip cells of *Ricinus communis L*.

Concentration (%)	Control	20	50	80	100
Mitotic Index (%)	95.51±1.300	96.45±0.930	86.04±0.772	67.99±1.798	52.22±0.831
Aberrant Cells(%)	0.000±0.000	0.023±0.023	1.420±0.381	6.95±0.504	13.26±0.381

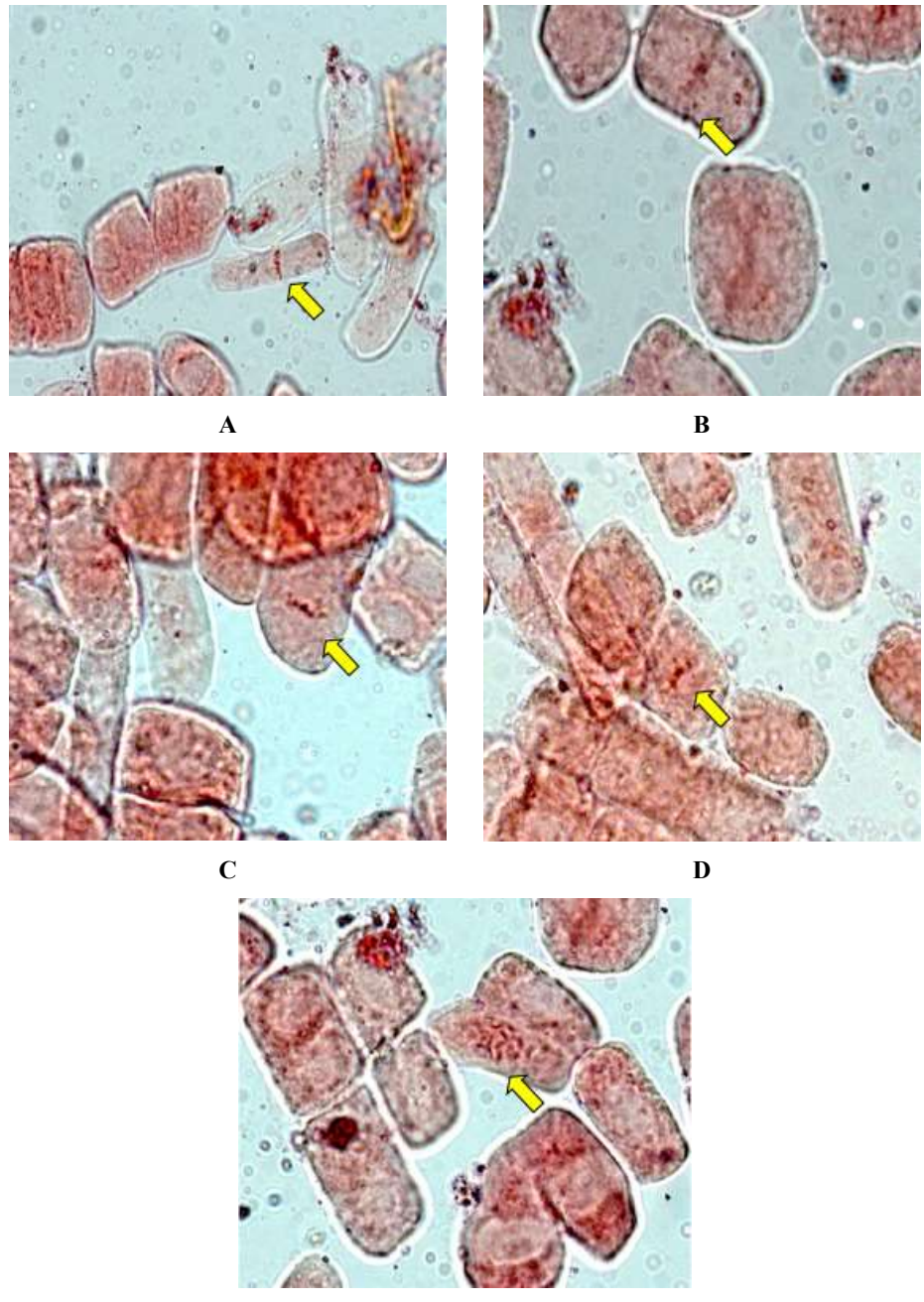


Fig. 6: Chromosomes of *Ricinus communis* L.A. Anaphase in control.
B. Metaphase in 20% fly ash.
C. Disturbed Metaphase in 50% fly ash.
D. Disturbed Metaphase in 80% fly ash.
E. Scattered chromosome in 100% fly ash.

tip cells. The study revealed that mitotic division increased in the presence of fly ash and created some abnormalities at higher concentrations. The percentage of the mitotic index decreased, and the percentage of aberrant cells increased with the higher fly ash concentration. The percentage of aberrant cells in control was negligible (Table 4). This indicates the presence of a certain cytotoxic or genotoxic substance in fly ash. The reduction in the mitotic index could be due to the inhibition of DNA synthesis or blocking in the G2 phase of the cell cycle, preventing the cell from entering mitosis (Sudha et al. 2018). No abnormalities were noted in Control and 20% fly ash concentration. While some abnormalities, like Disturbed metaphase and Scattered Chromosomes, were observed in the rest of the concentrations (Fig. 6). Disturbed metaphase was observed in 50% and 80% fly ash concentrations. Whereas, scattered chromosome was observed in 100% fly ash concentration. Disturbance during metaphase arises because of the effect of the treatment on the spindle that, leads to failure of the spindle mechanism. Chromosome scattering results from a prolonged metaphase arrest and is characterized by an uncoordinated loss of chromatid cohesion (Ananthakrishnasamy et al. 2009).

CONCLUSION

The present investigation indicates that the lower concentration of Fly Ash, i.e., 20% works as the enhancer for plant growth when mixed with pressmud, and the mixture is vermicomposted. The higher amount of Fly Ash can be toxic at the chromosome level, and hence it influences the overall growth of the plant.

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