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PHYTOGENOTOXICITY ASSESMENT OF MOSQUITO REPELLENT COILS USED IN PAKISTAN VIA PLANT CHROMOSOMAL ABERRATION ASSAY

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ABSTRACT

The rising prevalence of mosquitos borne deadly illnesses has made mosquitoes repellent usage common in Pakistan, with coils the popular choice. In order to estimate safe limits for humans three most commonly used type of mosquito repellent coils in Pakistan were compared for phyto-genotoxicity (DNA damage) using chromosomal aberration assay in Chickpea (*Cicer arietinum* L.) root tip cells. Chickpea seeds per treatment were incubated in coil smoke of five brands (PPJ, KRD, KCEP, GNA, TL) for 20, 40 and 60 minutes in approximately 24 by 24 inch Paper carton. Germination percentage was recorded by sowing seeds in sand pots. Genotoxic effects were assessed using a plant chromosomal aberration assay, measuring the Mitotic index, abnormality index and types of chromosomal aberrations were recorded to categorize different repellents. All the incubations of coils inhibit germination of chickpea seed as compared to positive control. The inhibitory effects on germination were more pronounced as the incubation increases except PPJ. For Mitotic index highly significant variation ($p \geq 0.01$) were found among all coils and their doses (20, 40, 60 minutes). KRD exhibited non-significant variations for only 60 minutes incubation. Decreasing trends for mitotic index with increasing doses was observed for most of treatments. For abnormality index highly significant variation ($p \geq 0.01$) were found among all the doses for KRD, where as PPJ and TL exhibited non-significant variations between 40 and 60 minutes incubation. Based on the Mitotic Index and abnormality index results, KCEP and KRD coils demonstrated highest genotoxicity. Therefore, these brands of coils should not be used and other brands may also be avoided

Keyword: mosquito repellent coils, Genotoxicity, Cytotoxicity, chromosomal aberrations,

INTRODUCTION

Pesticide mixture of substances intended for preventing pest, including vectors of human and animal disease (Stahl., 2002).

Pesticides can be broadly classified according to their intended target, herbicidal (Herb killing), larvicidal (Larvae killing) and insecticidal (Insect killing). Pesticides are used in agriculture as well as household (Kitchen Garden). Mainly household insecticidal pest are used for cockroach and mosquitoes.

Mosquitoes transmit Malaria, Yellow fever and Dengue to approximately 700 million people annually worldwide. With the advent of dengue epidemic people of Pakistan uses coils in lose rooms on daily basis (Mark, 2002; Patel *et al*., 2012; Gul *et al.*, 2013).

Although mosquito repellent manufacturers claim remarkable safety profile, their toxicities range from moderate hazards like skin and eyes irritation, low blood pressure to more severe problems including brain swelling in children, anaphylactic shock and neurotoxicity (Al-Saleh, 1994; Nerio *et al.*, 2010; Patel *et al.*, 2012; Gul *et al.*, 2013). All the mosquito coils are highly efficient in killing mosquitos due to ample amount of toxic chemical constitution (Tawatsins *et al.*, 2001; Yap *et al.*,1996; Yap *et al.*,1990).

The major active ingredients of the mosquito repellent are Pyrethroids, Prallethrin, pyrethrins, D-Allethrin, (Sinha *et al.*, 2004; Narendra, *et al.*, 2008; Kamble, 2012; Gul *et al.*, 2013). Mostly Coil repellent contain pyrethroid, prallethrin, pyrethrins, D-allethrin. Pyrethrins accounting for about 0.3–0.4% of coil mass (Lukwa and Chandiwana, 1998). When a mosquito coil is burned, the insecticides evaporate with the smoke, preventing mosquito from entering the room. Natural insecticide pyrethrin, which is obtained from the flowers heads of the plant *Chrysanthemum ciner ariaefolium*, some synthetic pyrethroids are also available in the market (casida,1980; Surgan *et al.*, 2002; Kamble, 2012)

Cytotoxicity: Cytotoxicity is the quality of cell destruction. It includes lose membrane integrity and rapid death of cells or cell lysis (Riss, and Moravec, 2004). As pesticides are basically poisons and are

designed to attack the immune, reproductive, or nervous system of mosquitos the major problems caused by cytotoxic effects (Farrell and Risau, 1994; Sinha *et al.,* 2006; Kamble, 2012; Patel *et al.,* 2012).

Allethrin a type-1-pyrethroid used as chief component of mosquito repellent. Due to prolonged and long-term use of allethrin is capacity of concern effects on human health (Mishra and Singh, 2003; Pankajand Prahlad, 2004).

Many people are allergic to mosquito repellents (Gul *et al.,* 2013). Short-term exposure to bio-allethrin has been reported to cause headache, nausea, dizziness, irritation of eyes, skin effect (Anonymous, 1991; Zhang *et al*., 1991; Leng *et al*., 1999) while

dermatitis, conjunctivitis, rhinitis-like symptoms, fatal hypersensitivity reactions affecting the respiratory tract are associated with Pyrethrins (Proudfoot, 2005; Panwer *et al.,* 2013). Epidemiologic studies have shown that long-term exposure to mosquito coil smoke can induce asthma and persistent wheeze in children (Azizi & Henry, 1991; Fagbule & Ekanem, 1994; Koo and Ho, 1994). Formaldehyde and acetaldehyde present in Mosquito coil smoke can induce sever upper respiratory tract complication (Chang & Lin, 1998).

Bioallethrin and D-allethrin can cause morphological changes in respiratory system and neurotoxicity (Cheng *et al*., 1992; Anonymous. 1991; Leng *et al*., 1999). Pyrethrins are of low chronic reproductive toxicity in animals and humans (Zhang *et al*., 1991). So the detailed and highly sensitive dose dependent cellular toxicity profile is generated using higher plant bioassay systems (Grant *et al.,* 1978). Failure of root germination is a major sign of cellular toxicity via inhibiting or altering root germination and growth (Noman *et al*., 2022; Noreena, 2024).

Genotoxicity: The deoxyribonucleic acid (DNA) damaging chemicals are called genotoxins (Galloway, 1994). The term **genotoxic agent** is applied for both physical and chemical agents that directly damage DNA, but may vary in level of damage (teratogenic to carcinogenic). The genotoxicity is an important consideration, because it has a potential to cause irreversible changes to genes and even cancer (M-boh, 2003).

Pesticide exposures leading to carcinogenesis was first correlated in humans three decades ago (Doll and Peto, 1981). Subsequent mouse based studies reported presence of Cytokinesis-blocked Micro-nucleus (MN) induced by prallethrin, a condition resulting by membrane formation to deleted arms of chromosomes during nuclear division (Victoria *et al*., 2013). More will be the frequency of MN greater will be the number of double stranded breaks to chromosomes resulting in different type of aberrant cells (Ayla *et al*., 2005). Particle size distribution of the coil smoke and identified a suite of volatile organic compounds (VOCs), including human carcinogens and suspected carcinogens (Liu *et al.,* 2003).

MATERIAL AND METHODS

Present study was conducted at Genetics Research Laboratory, Institute of Plant Sciences, University of Sindh, Jamshoro.

Plant Material and Incubation: 20 Chickpea seeds per treatment were incubated in coil smoke for 20, 40 and 60 minutes in approximately 20 by 24 inch Paper carton.

After incubation seeds were washed with distilled water dH_2O for 2 to 3 times. Untreated seeds were used as positive control and 0.3% Ethyl methanesulphonate (EMS) was used as negative control. Seeds were soaked in dH_2O for 60 minutes respectively than sown in the sand pots for root germination.

Germination percentage: The roots recovery was started after two days of sowing till last root emerged. The number of roots emerged are expressed in percentage.

Fixation*:* 2cm root sample were collected and fixed in solution of glaciered acetic acid and alcohol made in 3:1, for 24 hour.

Sample storage: Roots were transferred to 70% alcohol until used.

Slide preparation*:* Root tips were spread by using the squash technique (Dill and King 1983; Dill et al, 1986) slides were stained with 2% aceto-carmine.

Microscopy of slide*:* All the cytogenetic parameters were observed 6 slide per treatment with the help of microscope at 400 magnification, photographs were taken with digital camera (USB-2.0) dino eye

Figure. 1. mosquito repellent coil treatment of chick seed

Data analysis

Mitotic Index (M.I.):

Mitotic index was calculated as described by Racuciu (2009). It was calculated by following formula:

$$\mathbf{M.I.} = \frac{\textbf{Total dividing cells}}{\textbf{Total cells analyzed}} \times \mathbf{100}$$

Abnormal index (A.I.):

Abnormal index was calculated by the method of (Racuciu, 2009) according to following formula.

$$\mathbf{A.I.} = \frac{\textbf{Total abnormal dividing cells}}{\textbf{Total dividing cells}} \times \mathbf{100}$$

Means of mitotic index and abnormality index were further compared by Least Square Difference (LSD) test at $p \geq 0.01$ using computer software statistics 8.1.

RESULTS AND DISCUSSION

The results of germination percentage of mosquito repellent coils are compiled in Table.1 In 20 minutes incubation maximum percent increase was found in KCEP (13.3%) and maximum percent decrease was observed in GNA (6.6) while minimum decline was found in PPJ and TL (0%). In 40 minutes incubation no percent increase was found. Maximum percent decrease was recorded in GNA (35.29%) while minimum decline was found in KRD (23.52%). In 60 minutes incubation maximum percent increase was found in KRD (42.85) and maximum percent decrease was recorded in PPJ (14.28%), while minimum decline was found in GNA and TL (7.14%). The inhibitory effects of coil smock were more pronounced as the incubation increases except coil brand PPJ. Both coils exhibited dose dependent inhibition of germination compared to positive control except mosquito coil TL. Similar results were reported by Kanger *et al.*, (2014) that higher concentration of agriculture pesticide (Hexaconazole 5% EC and Triazophos 40% EC) can cause inhibition of germination. He also observed delay in germination in seeds treated with higher concentration of pesticide. Dubey and Fulekar (2011) applied five concentrations (10, 25, 50, 75 and 100 mg/kg), of three pesticide (Chlorpyrifos, Cypermethrin and Fenvalerate) and observed concentration dependent reduction and delay in seed germination of grass. Many researchers reported that long term use of allethrin a type-1-pyrethroid and chief component of mosquito repellent results in serious health problem in human (Pankaj and Prahlad, 2004; Kolaczinski and Curtis, 2004; Mishra and Singh, 2003).

The observed germination inhibition in the results results may be attributed to the presence of ample amount of chemicals (given on product label). Mosquito coil GNA contains 0.05% Prallethrin and PPJ also have Pyrethroid as active chemical (concentration not given in product label). KCEP, TL and KRD contain 0.20% D-Allethrin in common. While KCEP contains dried part of pyrethrum and KRD have with long lasting perfume which may be due to presence of parabens. Alteration in germination response is a clear indication of cytotoxicity of all the tested mosquito repellents applied.

The results of mitotic index and abnormality index are compiled in Table 5. Highly significant variation ($p \geq 0.01$) were found among all coils and their doses (20, 40, 60 minutes) for Mitotic index, while KRD exhibited non-significant variations for only 60 minutes incubation. For abnormality index highly significant variation ($p \geq 0.01$) KRD was found among all coils and their doses (20, 40, 60 minutes) where as PPJ (64.46) and TL (67.53) exhibited non-significant variation for 40 and 60 minutes incubation. Decreasing trends for mitotic index with increasing doses was observed for most of treatments except some brands of coil (PPJ, GNA, TL) and electric mosquito repellent the. Mitotic index is inversely proportional to the doses applied whereas abnormality index is directly proportional.

Present result are consistent with Amer *et al.* (1998) who investigated the ability of the two organophosphorus insecticides (Dursban and Dichlorvos) to induce chromosomal abnormalities in mouse spermatocytes. They observed increase in percentage of chromosomal aberration (19.3.+1.61, $P<0.01$ and 16.5_+ 0.63, $P<0.01$) with the increase in concentration of both insecticides after 24. Al-Ahmadi (2013) working with two organic insecticides (Kingbo and Azdar 10EC) observed decrease in number of normal cells and increase in the chromosomal aberration frequency in root tips of *Allium cepa* assay for 8, 16 and 24 h treatments. Ananthakrishnan *et al.,* (2012) who reported the genotoxic effect of different concentrations of agriculture pesticides, Furadan and Enodosulphan on (*Allium cepa*) root tips. He observed that when concentration and duration of treatment were increased, the decline in mitotic index and increase in percentage of chromosomal aberration.

The varying degree of mito-depreessive effects found in the study might be due to presence of different chemicals in each type of repellent like Pyrethroid, Bio-allethrin, D-allethrin, Pyrethrins, Prallethrin, Allethrin, which were present in mosquito coils, especially KCEP and KRD mosquito coils contain highl concentration of these chemicals which may lead to different chromosomal abnormalities. Renjana and Thoppil (2013) working on genotoxic effects of *Strobilanthes heyneanus* extract (0.5%) on *allium sepa* root recorded mitotic index values were decreased and increased abnormalities with increasing concentrations and longer treatment durations compared to the control ($p < 0.05$) in dose and time dependent manner.

Dizdari and Kopliku (2013) also observed phyto-genotoxic effect on *A. cepa* root meristematic cells exposed to three concentrations of diclofop-methyl (herbicide) and lindane (insecticide). They observed decrease in dividing cells in all concentrations and abnormal cells were increased. Similar trends were

observed by Grage and Grage (2004) in mosquito coil containing allethrin as active ingredient. Similarly, Kamble (2012) reported selective damage to lung and liver due to inhalation of mosquito repellents containing allethrin 0.88% (Good Knight) by rat. Sinha *et al*. (2006) suggested that Pyrethroid-based mosquito repellent inhalation during early development period of human fetus may have adverse effect on developing nervous system.

The most frequent abnormality induced by all brands and doses of mosquito repellent coils was fragmentation and sticky metaphase while least abnormalities were laggard, anaphase bridges, translocation rings, micro nucleli, di-nuclei and multi-nuclei. Working on pesticide β -cyfluthrin induced genotoxicity in male Swiss albino mice Verma *et al.* (2013) found multiple chromosomal aberrations like acentric fragments, chromosome breaks, centric rings and polyploidy. Al-Ahmadi, (2013) investigated two organic agricultural insecticides (Kingbo and Azdar 10EC) using root tips of *Allium cepa* assay. Most frequent abnormalities observed by him were stickiness, disturbance, c-metaphase, chromosome bridges in anaphase and telophase, lagging chromosome and micronuclei appearing in interphase cells; while s- metaphase, s-anaphase and fragments were least common type of chromosomal aberrations. Renjan aand Thoppil (2013) found similar abnormalities in *Allium cepa* root when subjected to methanolic extract of (*Strobilanthes heyneanus*) Nees (0.5%). The major clastogenic abnormalities found by them were nuclear and chromosome lesions, chromosome pulverization, anaphase bridges, fragments, stickiness, *etc.* Kripa *et al*., (2013) reported occurrence of micronuclei (MN) in South Indian human volunteers subjected to prallethrin based mosquito repellents.

Zeljezic and Vrhovac (2001) also found significant increase in number of aberrant cells with the increase in exposure time utilizing alkaline single cell gel electrophoresis (comet) essay in peripheral blood lymphocytes. Present results are in agreement with Yuzbaşioğlu *et al*. (2009) and Ananthakrishnan *et al.* (2012) who reported variations in DNA damages with respect to dosage and exposure of time of pestisidse in *Allium cepa*. Saxena and Saxena (2010) also reported that mosquito coils smoke generate free radicals which cause oxidative damage resulting hepatic injury. Verma *et al.* (2013) reported that cyfluthrin induced oxidative stress in mice. (GR) activity. Lusio *et al*., (2014) reported concentration- and time-dependent genotoxic damage by mixture of permethrin and allethrin in human peripheral blood lymphocytes (PBL) in vitro.

Recently histological tissue-alignment alterations of lung and liver model followed by modification of vital health indicators in mouse has been witnessed by (2020).

Akbar *et al.* (2012) also observed Pyrethroids (permethrin and fenvalarate) induced oxidative stress in *H. armigera* in a time- and dose-dependent manner resulting in increased Lipid peroxidation, H_2O_2 content, and lactate dehydrogenase (LDH) activity and increased glutathione reductase.

The overall results of current plant cell-based studies clearly indicate the possible cellular as well as genetic damages to human cells. Deletions are the most common type of DNA damage in different cancers and specially breast cancer.

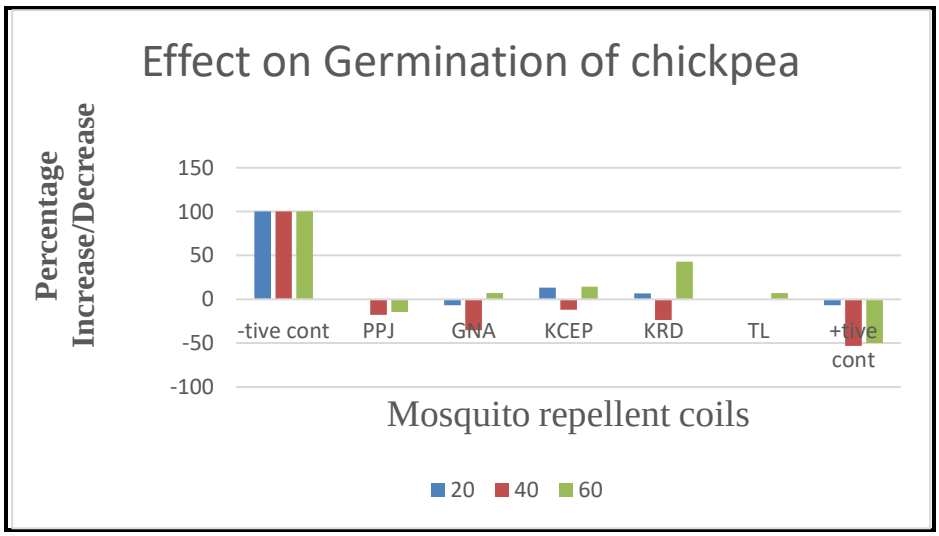

Figure 2 Effect of different incubation times of mosquito repellent coils on germination percentage of chickpea. (PPJ= power plus jumbo; GNA= goodnight advanced; KCEP=king coil extra power; KRD= king red dove; TL= tyfon lanvender)

Table 1 Effect of mosquito repellent coils on Abnormality index (A.I.) and Mitotic index (M.I.) in chickpea root tip cells

S. No.	Name of treatment	M.I (%)			A.I (%)		
		20m	40m	60m	20m	40m	60m
1.	-tive control	100^{a}	100^{a}	100^{a}	0^{f}	0^{f}	0^{f}
2.	PPJ	96.69^{ab}	62.73^{cd}	65.41^{b}	61.97^{d}	64.46^{e}	82.01^{d}
3.	GNA	79.91^{b}	58.04^{cd}	60.70^{c}	74.87^{c}	67.53^{d}	80.58^{d}
4.	KCEP	94.23^{ab}	70.33^{b}	58.98^{c}	78.16^{b}	81.92^{c}	96.70^{b}
5.	KRD	99.32^{a}	95.68^{a}	59.33^{c}	98.64^{a}	95.49^{b}	92.03^{c}
6.	TL	96.64^{ab}	63.77^{c}	67.10^{b}	60.61^{d}	$65.30d^{e}$	67.53^{e}
7.	+tive control	71.42^{c}	56.84^{d}	40.3^{d}	44.44^{e}	98.79^{a}	100^{a}

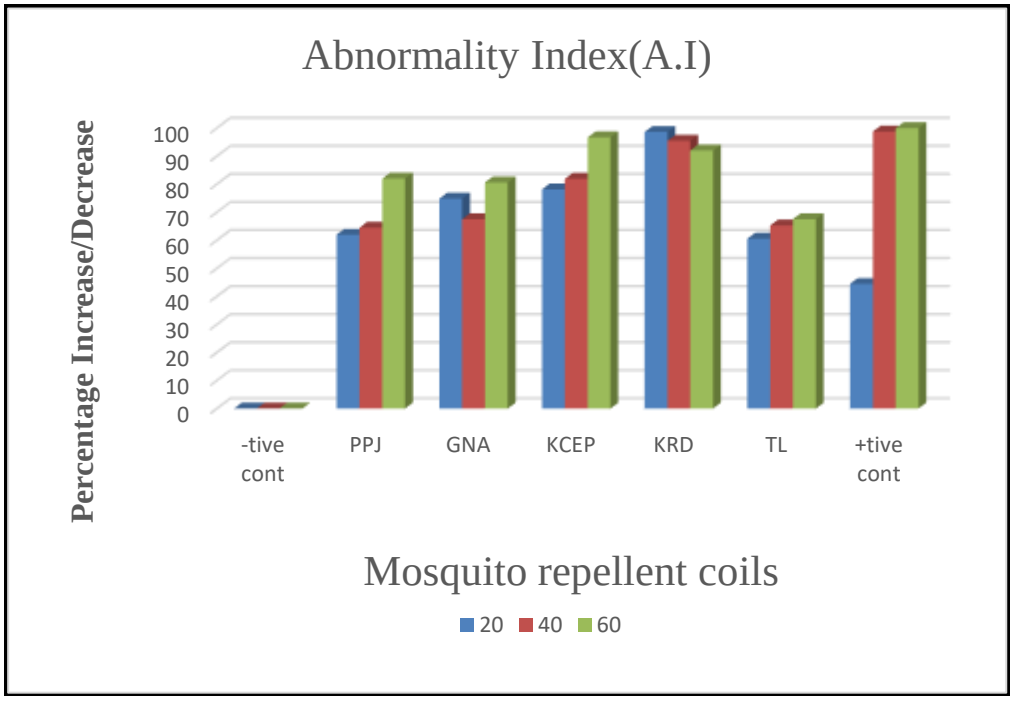

Figure. 3. Effect of mosquito repellent coils on Abnormality index

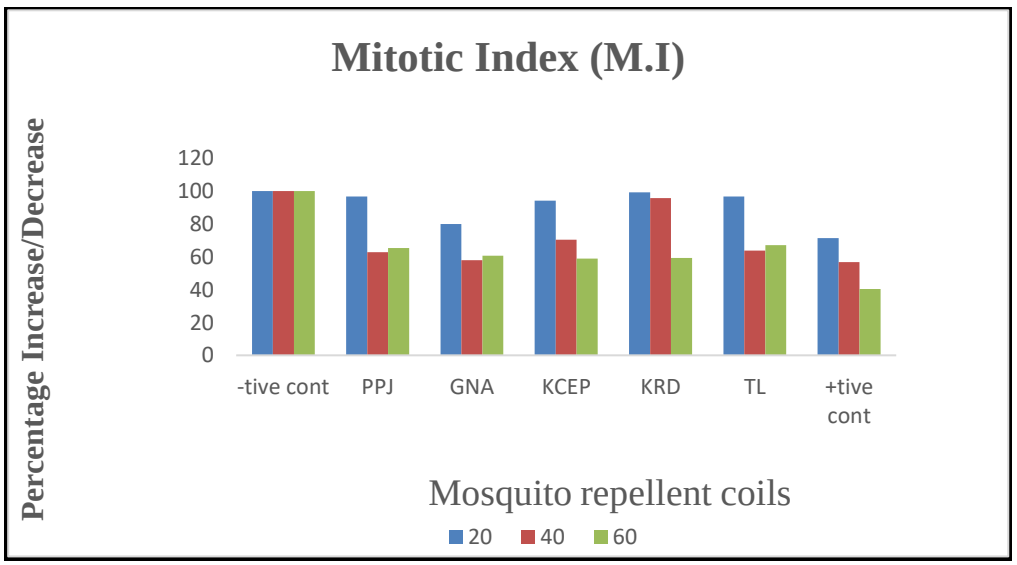

Figure 4 Effect of mosquito repellent coil on mitotic index

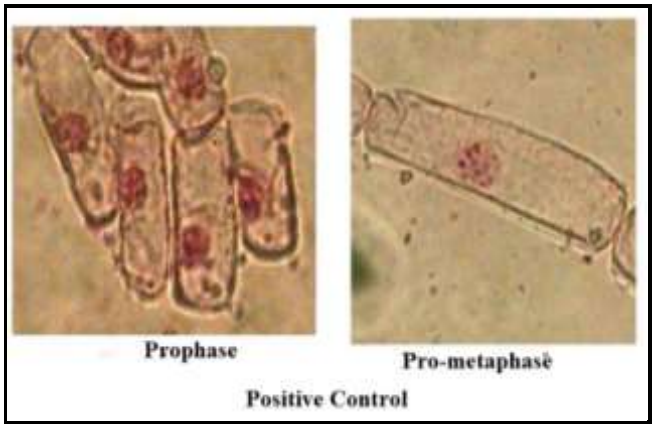

Figure. 5. Normal cells of positive control

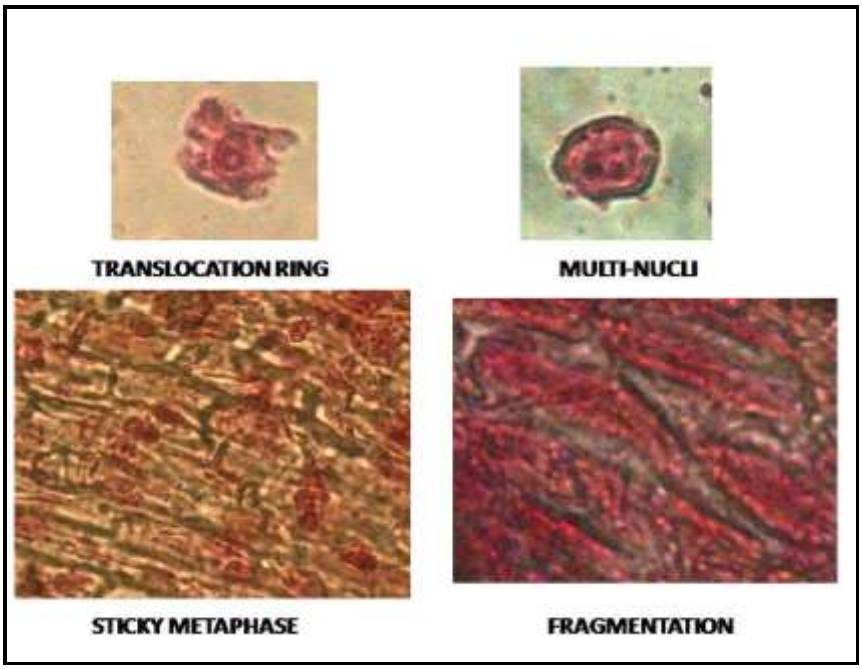

Figure. 6. EMS induced chromosomal aberrations

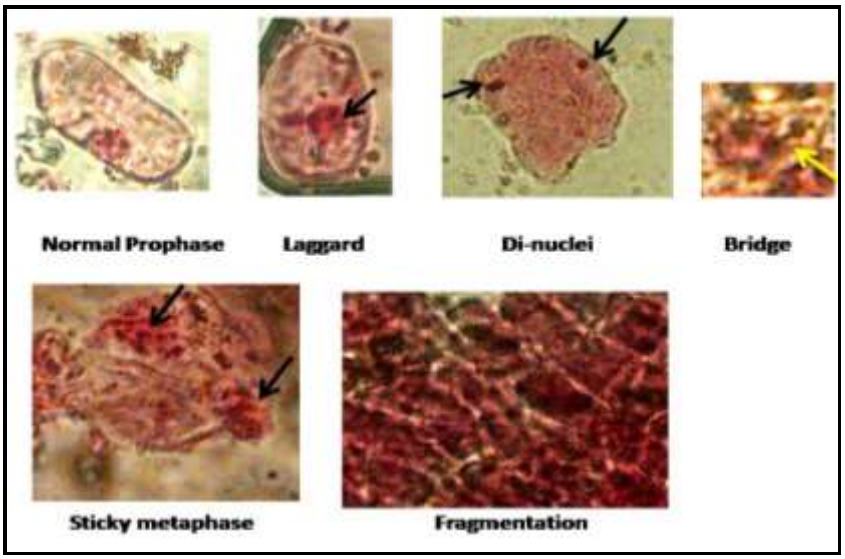

Figure. 7. PPJ induced chromosomal aberrations

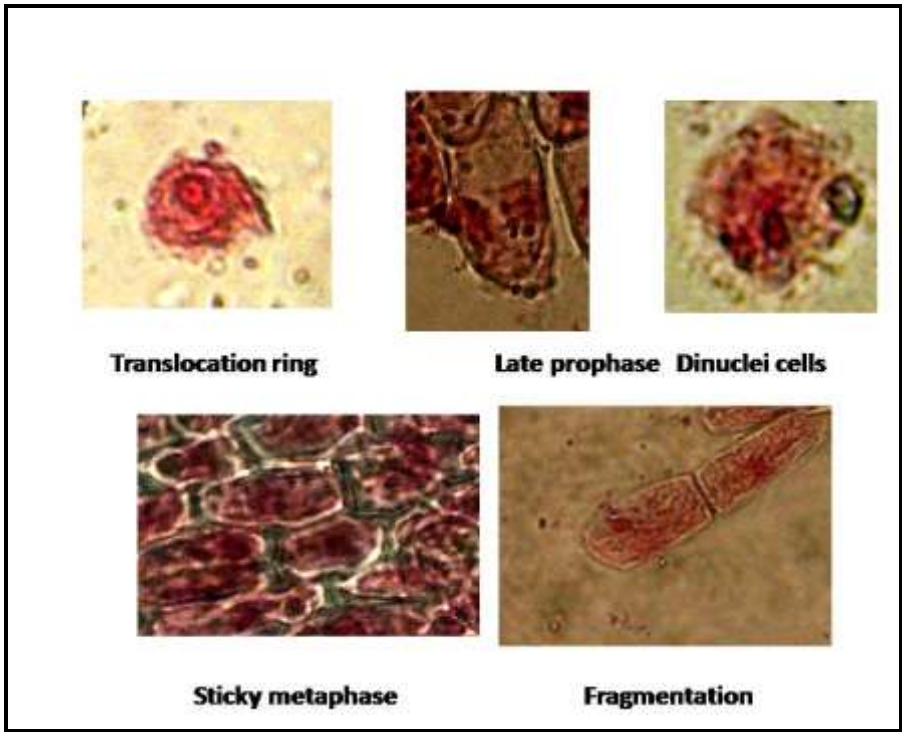

Figure 8 GNA induced chromosomal aberrations

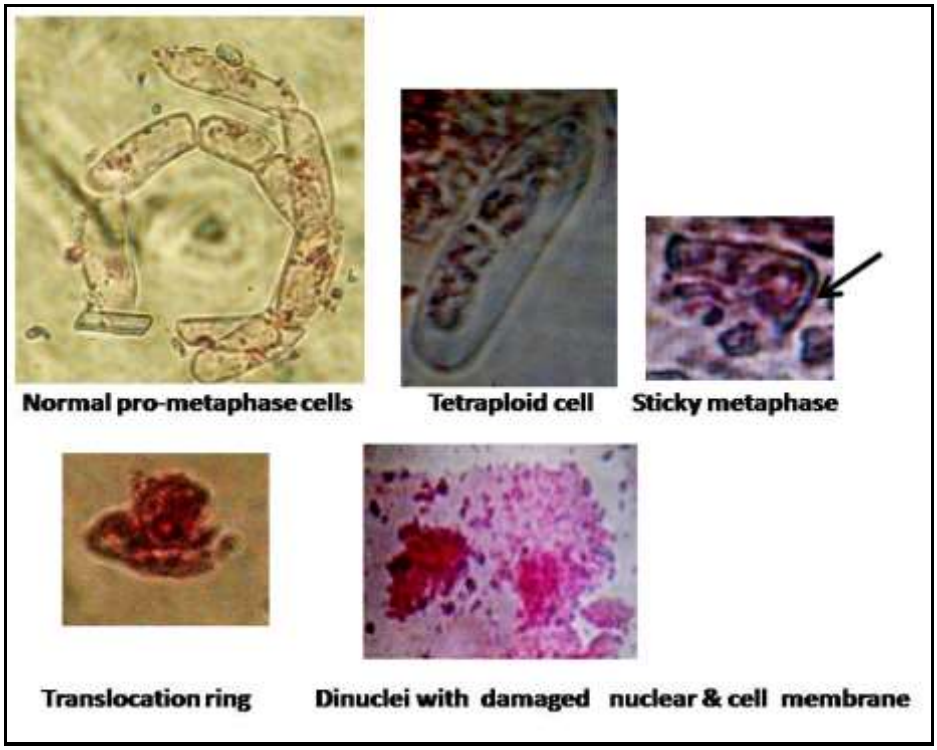

Figure. 9. KCEP induced chromosomal aberration

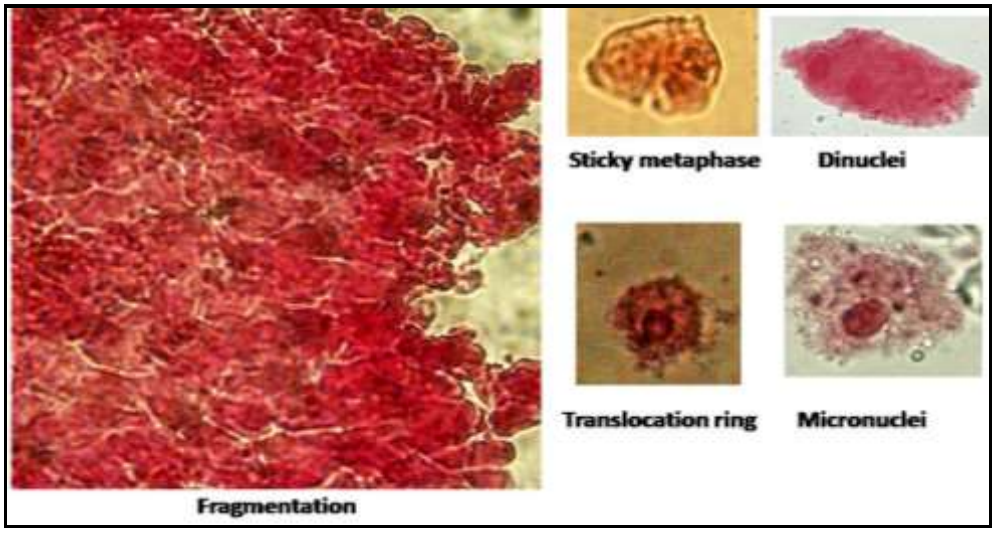

Figure. 10. KRD induced chromosomal aberration

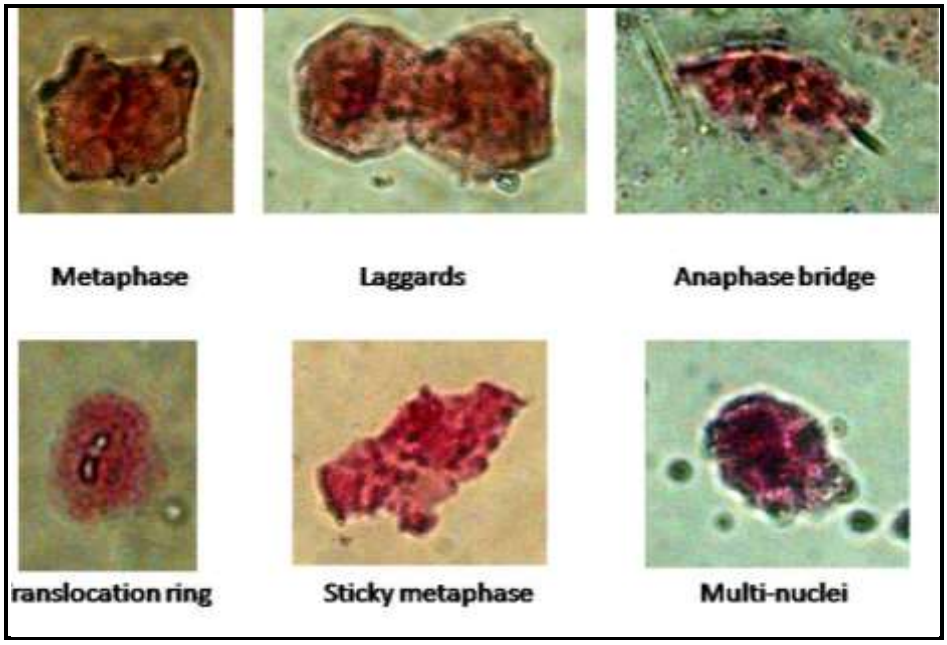

Figure. 11. TL induced chromosomal aberration

CONCLUSION

Alteration in germination response, mito-depression and deletions leading to various types of chromosomal aberrations are clear indications of phyto-genotoxicity and carcinogenicity of applied coil brands. Based on abnormality index (A.I) profile, KCEP and KRD were identified as the most genotoxic brands. Therefore, these coils brands should be avoided and other brands should also be used sparingly.

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