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CYTOGENETIC EFFECTS OF ^{48}TITANIUM (^{48}TI) ON MERISTEMATIC CELLS OF ROOT TIPS OF *LENS CULINARIS* MED.

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Abstract

Cytogenetic effects of ^{48}Titanium (^{48}Ti) on meristematic cells of root tips belonging to the plant (*Lens culinaris* Medik.) have been Investigated. Seeds of the plant, prepared were kept in ^{48}Ti standart for different time period as control during 1/4, 1/2, 1, 2, 4, 8, 12, 16, 20, 24 hours. Seeds treated with ^{48}Ti were made sprout and the root tips obtained were prepared for microscopic examination. At the end of the microscopic examinations, some abnormalities as chromosome breakings, chromosome dispersion, bridge chromosome, chromosome adherence, ring chromosome were observed. Abnormalities were seen at each treatment depended on the time periods. Variety and number of abnormality were usually seen to be increasing, depending on the increase of treatment time. The results obtained were evaluated statistically.

Introduction

^{48}Titanium (^{48}Ti) with atomic number 22 is a lightweighted, strong, bright, corrosion-resistant transition metal. The most useful properties of titanium in metal form are being resistant to corrosion and having high strength-weight ratio among all metals. It is well suited to the role because it emits readily detectable 115700 keV gamma rays, and its half-life is less than a minute. About 95% of titanium ore extracted from the Earth is used for production of titanium dioxide (TiO_2). The Ti distribution in soil profiles depends mainly on the geological processes and on soil-forming processes (Gaworek, 1990). In following years, even though knowledge of the soil and intra-species effects impacting on ^{48}Ti transfer has become extensive, there is still no systematic understanding of inter-species factors cauing plant uptake of ^{48}Ti. In terrestrial plants, titanium accumulates in a species-specific and organ-specific pattern, with increased concentrations in senescencing leaves. Dumon & Ernst (1988) also emphasize that titanium fertilizer, applied through roots or leaves in growth experiments stimulates plant growth in a species-specific manner. It can stimulate chlorophyll content, enzyme activities and uptake of major and minor nutrients. The implications of advances in soil science and molecular biology to soil and intra-species effects have been reviewed, but there have been few analyses of inter-species effects. A phylogenetic perspective has been focused on inter-species effects in soil-to-plant transfer of inorganic contaminants including metals ^{137}Cs, ^{36}Cl, ^{90}Sr , ^{109}Ru , ^{35}S and ^{60}Co, but has not been reported enough data for ^{48}Ti (Willey & Fawcett 2005; 2006). Such an analysis of inter-species effects on ^{48}Ti transfer could complement established knowledge of soil and intra-species effects on the soil-to-plant transfer of ^{48}Ti and other radioisotopes (Willey & Wilkings 2008). As a soil-to-plant transfer, all the treatments containing titanium increase the tree performance (branch elongation, flowering and fruit setting intensities) and fruit size. While harvested fruits from the Ti-treated trees show improved resistance to compression and penetration, there is a decrease in weight-loss during postharvest storage (Alcaraz-Lopez 2003). Schmidtke *et al*., (2004) have studied 3 morphologically different varieties of lentil (*Lens culinaris* Medik.) and one cultivar of naked spring barley (*Hordeum vulgare* ssp. *nudum* L.) as monocrops and substitutive mixture. There have been several studies about titanium and other metals cytogenetic effects (İnceer *et al.*, 2003; Kıran & Şahin 2005). Janas *et al*., (2010) have investigated cytogenetic effects of copper ions on root cells of *Lens culinaris* (Medik.). They detected that this compund was accumulated particularly in vacuoles and the cell wall. Li *et al*., (2010) made contributions about the intake and distribution of some radioactive elements by plants. Çelik *et al*., (2004), Kabata-Pendias (2004), and Munzuroğlu&Geçkil (2002) and Wojcik *et al.*, (2010) Saeed *et al*., (2010) have focused on the effects of heavy metal pollution on plants, resulted from different factors at environment and enterence of these elements into soil and plant. Çanakçı & Karaboğa (2013) investigated that the effect of cadmium on the cucumber (*Cucumis sativus* L.). Ismail *et al*., (2013) evaluated that the toxicity effects of heavy metals (lead and cadmium) on germination, root length and dry biomass of tree species *Thespesia populneoides*, *Leucaena leucocephala* and *Delonex regia.* Salam *et al*., (2011) observed that sodium chloride tolerance in rice (*Oryza sativa* L.).

In this study, the effects of ^{48}Ti treatments in different time periods on root tip cells of *Lens culinaris* were investigated.

Material and Methods

Seeds of *Lens culinaris* were used in the study. Plump, sound and equal sized seeds were chosen and kept in %10 sodium hypochlorite for 10 minutes. So, contamination of seeds was prevented. Then, seeds were washed by distiled water 5-6 times and were dried on filter papers at 25 ºC. As a ^{48}Ti source, TiO_2 with standard at 1 M was prepared with 500 ml distiled water. Seeds were kept within both ^{48}Ti standard for control during 1/4, 1/2, 1, 2, 4, 8, 12, 16, 20, 24 hours. Then, seeds were washed by distiled water and germinated in petri dish at 20–25ºC. After the fixation of root tips obtained, they were put in 70% ethyl alcohol. Stock root tips were stained by Feulgen method] and were got ready for microscopic examination. Homoligous areas were chosen on these preparations for cytogenetic

examination; the cells in these areas were counted and the number of mitotic cells were also detected Darlington & La Cour (1976). Chromosomal abnormalities were tried to detect in the cells counted. Preparates was photographed with motorized Leica DM 3000 microscope.

Results

At the end of the study, it has been observed that ^{48}Ti standard treatment on the seeds at different time periods increased mitotic cell division. This situation was reached on the top point at 1/4 hour of treatment. At the 1/2, 1, 2 and 4 hours of treatment, mitotic cell division was decreased. Mitotic division was increased again at the 8 hour of treatment and it was decreased again at the 12 and 16 hour of treatment. Mitotic division was increased again at the 20 and 24 hour of treatment. Mitotic cell division was observed low level all treatment time according to control group (Table 1; Fig. 1).

In the cells of the root tips of treated with ^{48}Ti Investigated seeds various chromosomal abnormalities as fish bones chromosome adherence, chromosome dispersions, chromosomal adherence, bridge chromosome, chromosome breaking, chromosome shrinking, ring chromosome at different stages of mitotic division were detected.

The most observed abnormality was chromosome dispersion. Fish bones chromosome adherence, chromosome dispersions, chromosomal adherence, bridge chromosome and shrinking chromosome was observed all treatment hours. Chromosome dispersions were observed high level at 8, 12, 16, 20 and 24 hour treatment time. Chromosome breaking was seen all treatment times except to 1/2, 24 hour treatment. Fish bones chromosome adherence abnormality was determined the highest level at 20 hour treatment. Chromosome adherence was observed highest level at 16, 20 and 24 hours of treatment. Bridge chromosome was determined high level at 16 hours treatment. Chromosome breaking was observed high level at 1/4, 12 hour of treatment time. Ring chromosome was observed 1/4 and 1/2, hour treatment. Chromosome shrinking was seen all treatment times (Table 3; Figs. 1-12) In addition, the germination percentages of the seeds treated with ^{48}Ti given in the Table 2 were calculated. In all treatment periods, the percentage of germination is close to the germination percentage of the control group and ^{48}Ti were reported to speed the (Table 2).

Table 1. The mitotic index of root tip cells of *Lens culinaris* at the period of different time.

Time (hour)	Mitotic index ± *S.D.	Time (hour)	Mitotic index ± *S.D.
¼	15.25 ± 4.62	8	10.12 ± 2.60
½	11.17 ± 4.07	12	9.59 ± 2.19
1	11.66 ± 3.08	16	7.79 ± 1.66
2	8.77 ± 2.95	20	10.86 ± 2.81
4	8.32 ± 1.60	24	11.43 ± 1.81
Control group	16.25 ± 3.78		

*S.D: Standard deviation

Table 2. Seed germination ratio (%).

Time (Hour)	Germinated seeds (%)	Time (Hour)	Germinated seeds (%)
¼	94.45	**8**	95.24
½	92.79	**12**	92.86
1	95.04	**16**	93.59
2	93.21	**20**	93.84
4	94.76	**24**	95.35
Control group	98.86		

Table 3. **Chromosome abnormality on the root tip cells of *L. culinaris.***

		Investigated abnormality (%)						
Dose treated (M)	**Treatment time (hour)**	**Fish bones**	**Chromosome dispersion**	**Chromosome adherence**	**Chromosome breaking**	**Bridge chromosome**	**Chromosome shrinking**	**Ring chromosome**
1	1/4	28.56	14.28	7.14	21.42	21.42	7.14	14.28
1	1/2	9.20	18.40	9.20	0	9.20	9.20	18.40
1	1	17.74	17.74	17.74	8.87	8.87	8.87	0
1	2	23.81	11.90	11.90	11.90	11.90	23.81	0
1	4	22.25	11.12	22.25	11.12	11.12	11.12	0
1	8	9.28	27.83	18.55	9.28	18.55	9.28	0
1	12	31.14	31.14	41.52	20.76	20.76	10.38	0
1	16	26.00	52.00	39.00	13.00	39.00	26.00	0
1	20	38.32	28.74	38.32	9.58	9.58	19.16	0
1	24	17.63	26.45	26.45	0	8.82	8.82	0

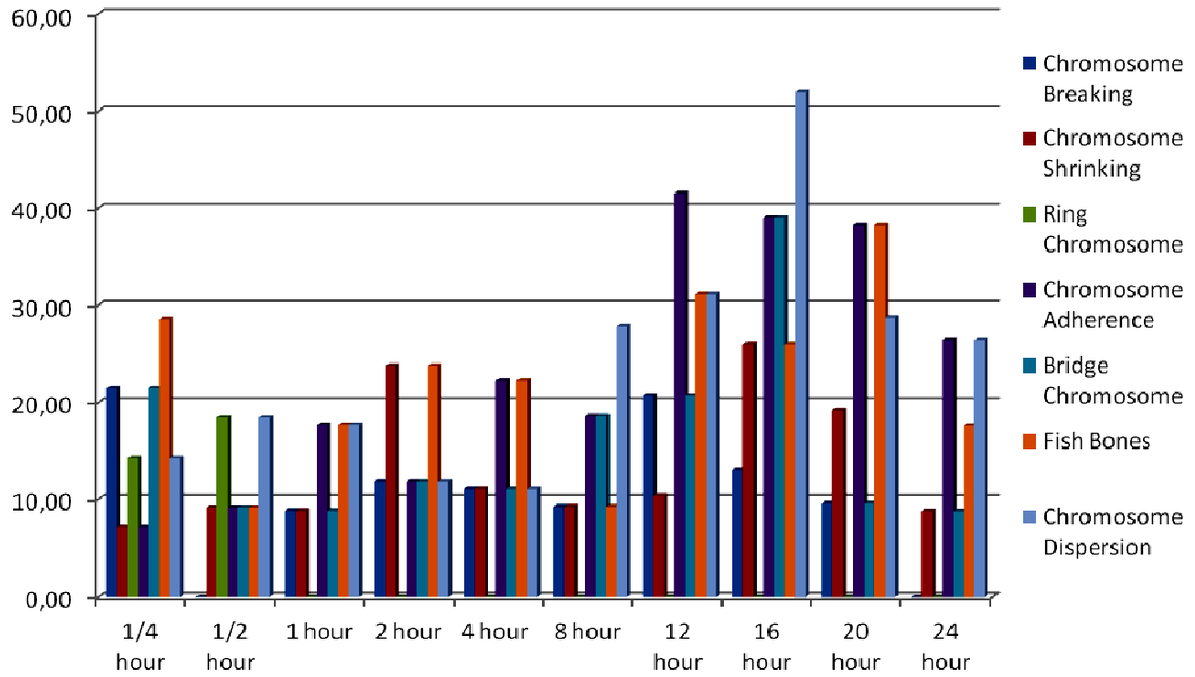
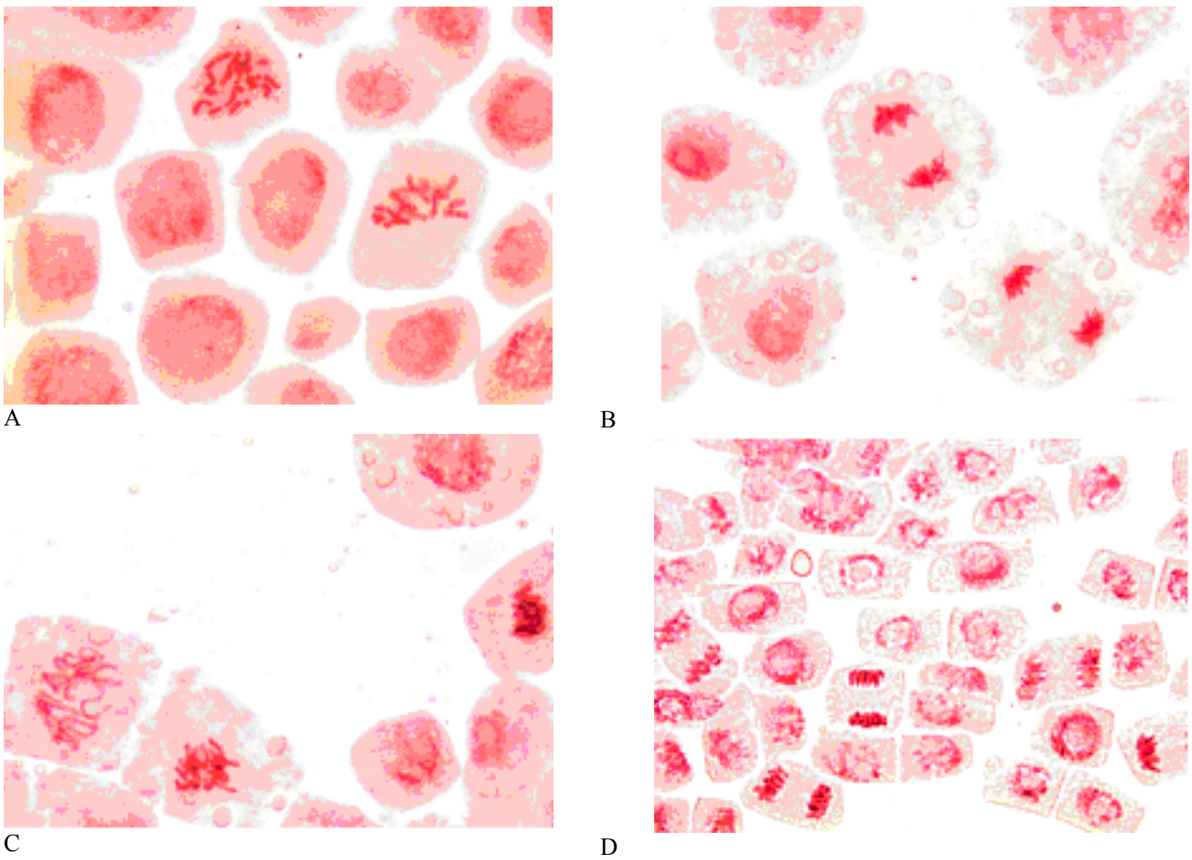


Fig. 1. Chromosome abnormality on the root tip cells of *L. culinaris*.

Discussion

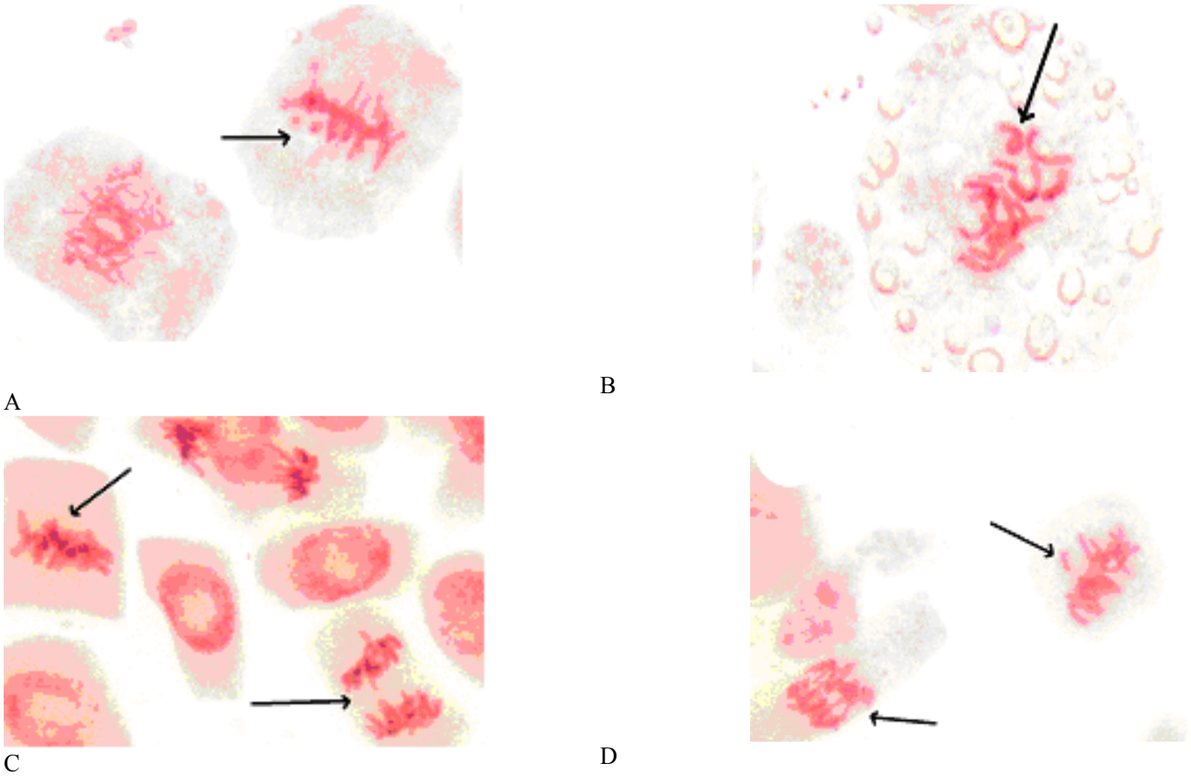
In present study, cytogenetic effects of ^{48}Titanium (^{48}Ti) on meristematic cells of root tips belonging to the plant (*Lens culinaris* Medik.) have been Investigated. Seeds of the plant, prepared were kept in ^{48}Ti standart for different time period as control during, 1/4, 1/2, 1, 2, 4, 8, 12, 16, 20, 24 hours. It was determined that ^{48}Titanium (^{48}Ti) was caused to some abnormality as fish bones chromosome adherence, chromosome dispersions, chromosomal adherence, bridge chromosome, chromosome breaking, chromosome shrinking, ring chromosome. Similarly, copper chloride has caused to some chromosamal abnormality on root tip cells of *Vicia hirsuta*. The most observed abnormalities were chromosome adherence and bridge chromosome (İnceer *et al*., 2003). In other study, the results pointed out that increase of the lead ($PbCl_2$) concentrations cell division was decreased, several mitotic anomalies such as c mitosis, lagging chromosomes, multipolar anaphases and chromosome bridges on root tip cells of lentil (*Lens culinaris* Medik.) (Kıran & Şahin 2005). In similar, the germination rates of uranium exposed *Vicia faba* seeds were not inhibited by the uranium tailings lixivium. The growth of seedling was considerably stimulated by uranium tailings at low concentrations rather than at high concentrations. The results revealed that nuclease (RNase) activity were stimulated at low uranium tailings lixivium concentrations and inhibited at high concentrations. As the concentrations of uranium tailings lixivium became higher, the inhibitory effect increased. So, the uranium tailings have complex effects on nuclease activity (Yi, *et al*., 2007).In terms of cytological effects of the insecticide Phosdrin (mevinphos) and the herbicide Bladex, the root tips of *Tradescantia* and *Vicia faba* were observed and compared with those of the chemical mutagen ethyl methane sulfonate (EMS). In addition, plants of *Vicia faba* were sprayed prior to floral initiation and pollen mother cells examined for chromosomal abnormalities. It has been determined that the frequency of mitotic cell division were affected by uranium depending on the different treating time and uranium led to chromosomal abnormalities in the *Vicia faba* cells (Özdemir *et al*., 2008). On the other hand, some of scientist were applied the *Allium cepa* test to estimate the impact on plant chromosomes of nuclear pollution in the inhabited zones of the Ukraine. The result pointed out the raise of radioactive metal concentration in the *Allium cepa.* (Kovalchuk *et al.,* 1998). In another study, it was determined that effect of the cadmium chloride in the pure germ line of broad bean (*Vicia faba* L.) were evaluated in relation to the chromosomal abnormalities and rate of cell division. Seeds grown in the nutrient medium for 48 hrs containing differet concentrations of cadmium chloride showed different genotoxic effects such as polyploidy, multipolarity, chromosomal bridge with fragments, lagging chromosome and micronuclei. Relative division rate (RDR) was decreased with increasing cadmium concentration (Parween *et al*., 2011).

Radioactive elements can pass to plants through soil and then can reach people. Studies about the role of transition metals in soil and plants are not sufficient enough (Kasianenko & Kulieva 2002). There are reports about copper, zinc, lead and chrome that pointed out the result of causing to clastogenic effect on *Allium cepa* root tips (Arambasic *et al*., 1995). Leonard *et al*., (1983) reported that mercury compounds were effect spindle fibers at the time of cell division on the plant as *Vicia faba* and *Allium cepa*. There was a determination that titanium was functioned like mercury which acts as an inhibitor which blocks to protein syntesis and cause to mitotic lagging (Carvajal *et al.*, 1994). In this study, we intended to determine cytogenetic effect of ^{48}Titanium (^{48}Ti), arising from transition (heavy) metals, on the root tip cells of *Lens culinaris* Medik.



A B C D

Fig. 2. Investigated chromosome abnormality (control group) normal cell division.



A B C D

Fig. 3. Investigated chromosome abnormality (1/4 hour) C, D: bridge chromosome, A, D: chromosome breaking, B: ring chromosome C: fish bones chromosome adherence.

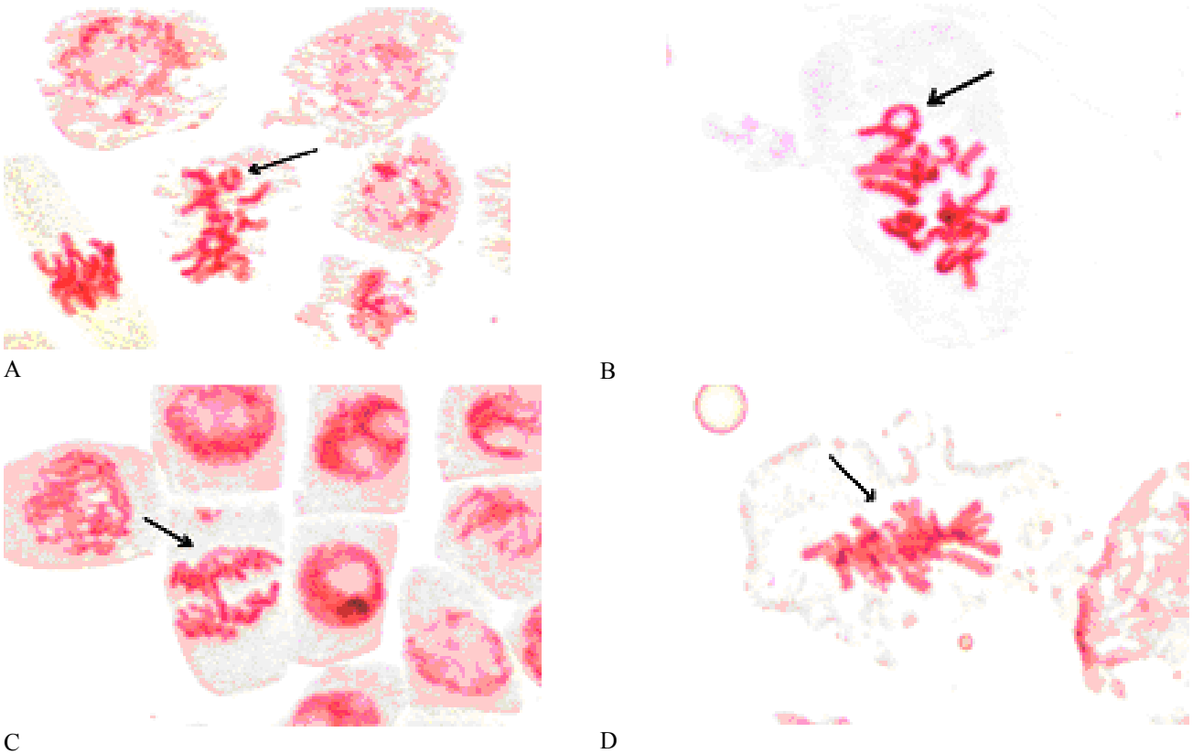


Fig. 4. Investigated chromosome abnormality (1/2 hour) C: bridge chromosome, A, B: ring chromosome, D: fish bones chromosome adherence.

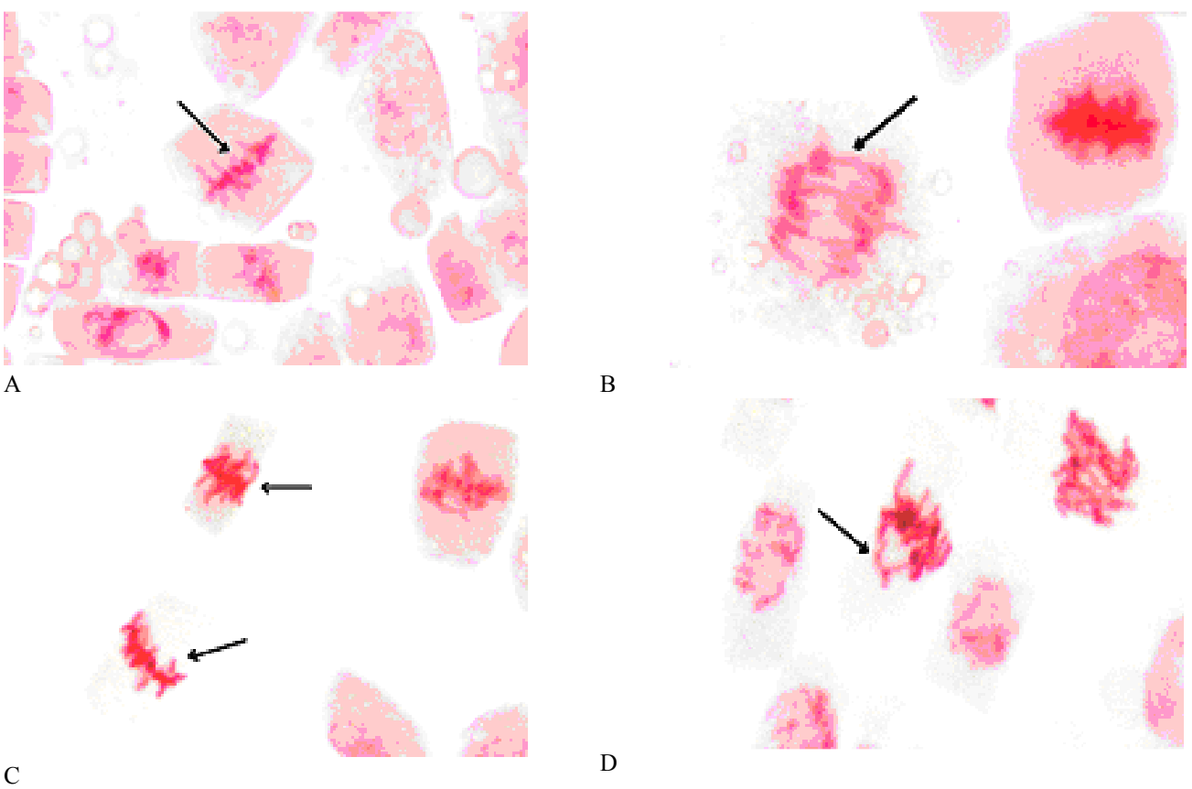


Fig. 5. Investigated chromosome abnormality (1 hour) D: chromosome dispersions, B: bridge chromosome, C: chromosome breaking, A: fish bones chromosome adherence.

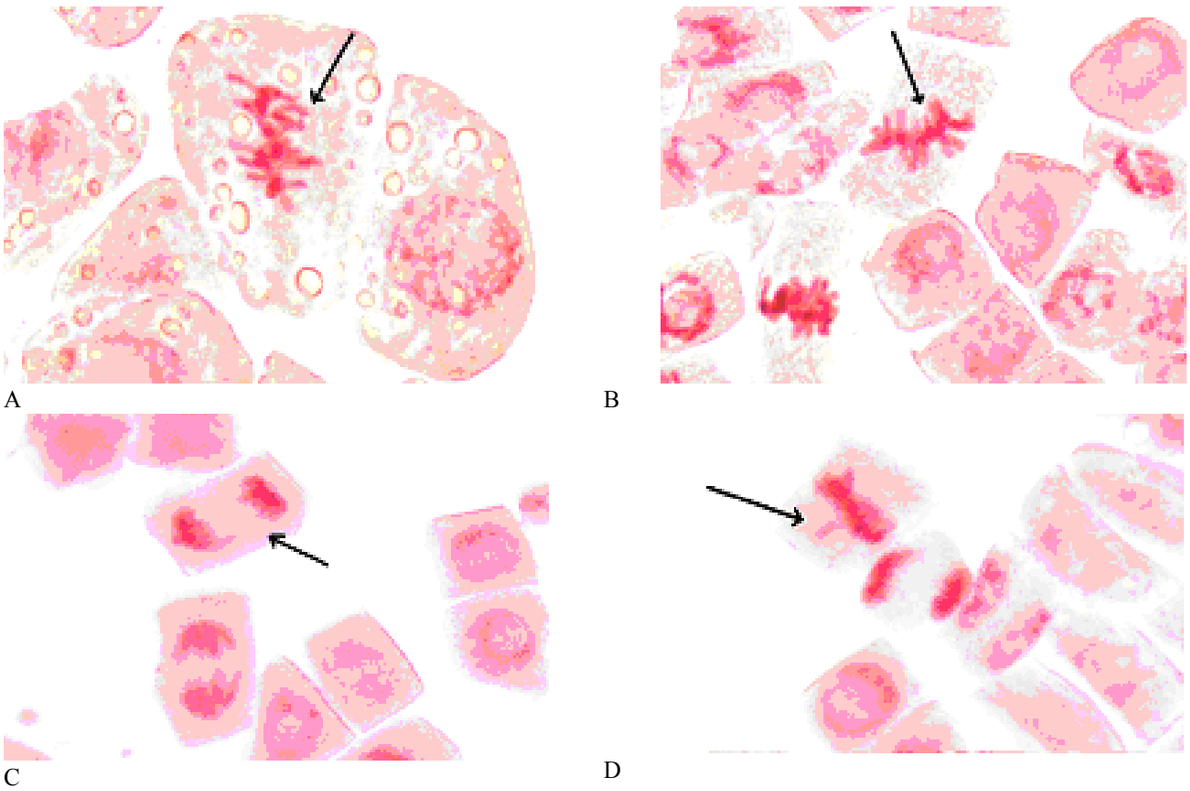


Fig. 6. Investigated chromosome abnormality (2 hour) D: chromosome breaking C: bridge chromosome, A, B: fish bones chromosome adherence.

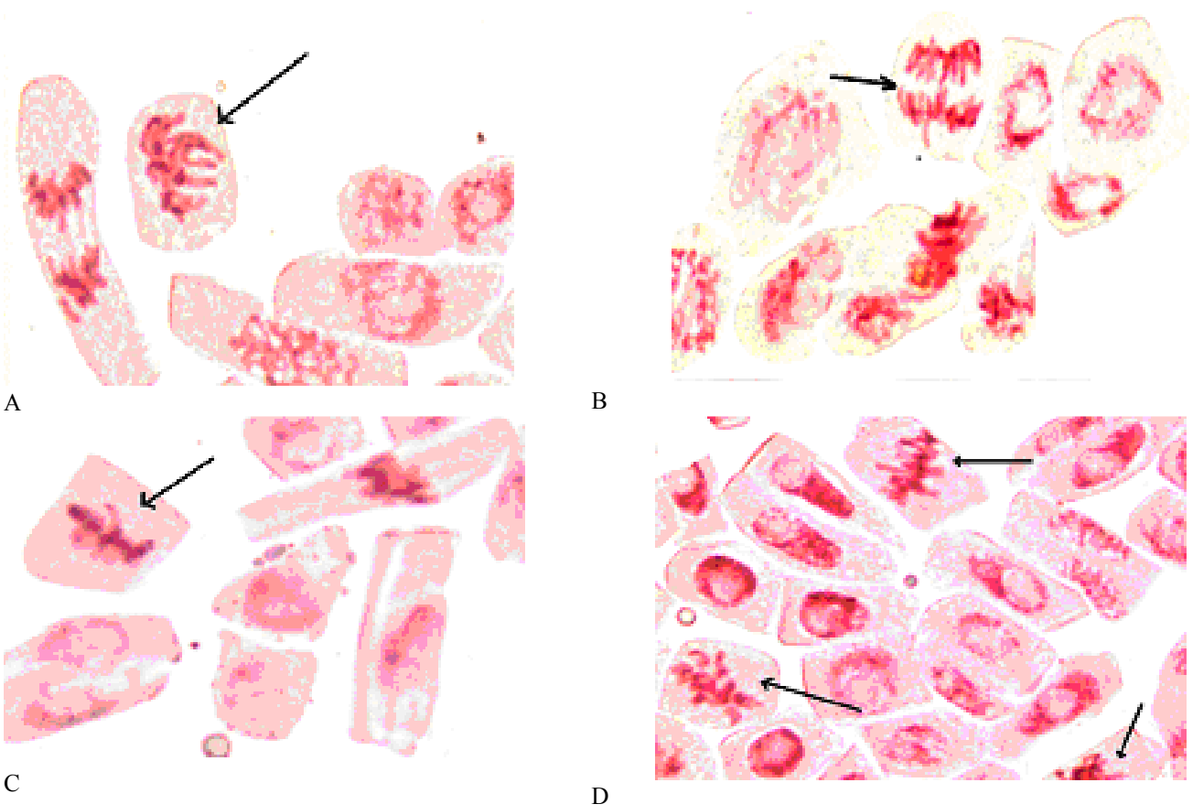
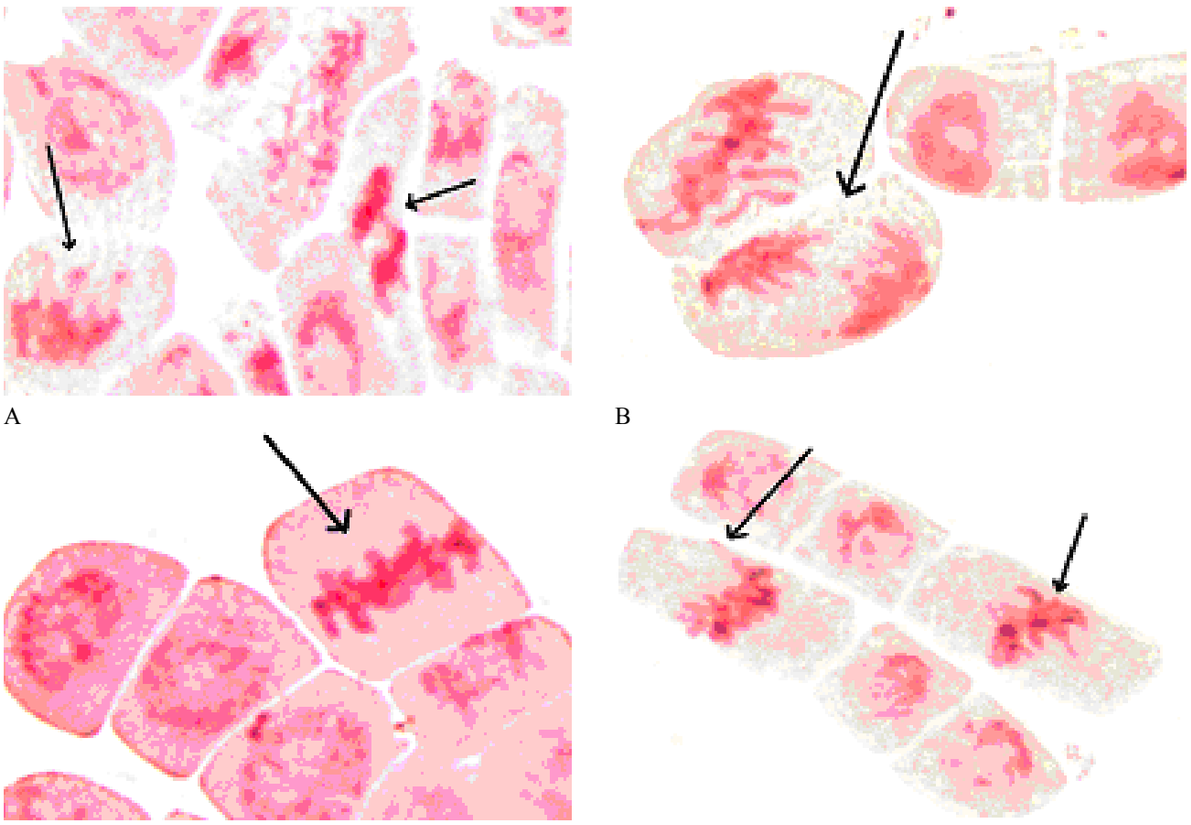
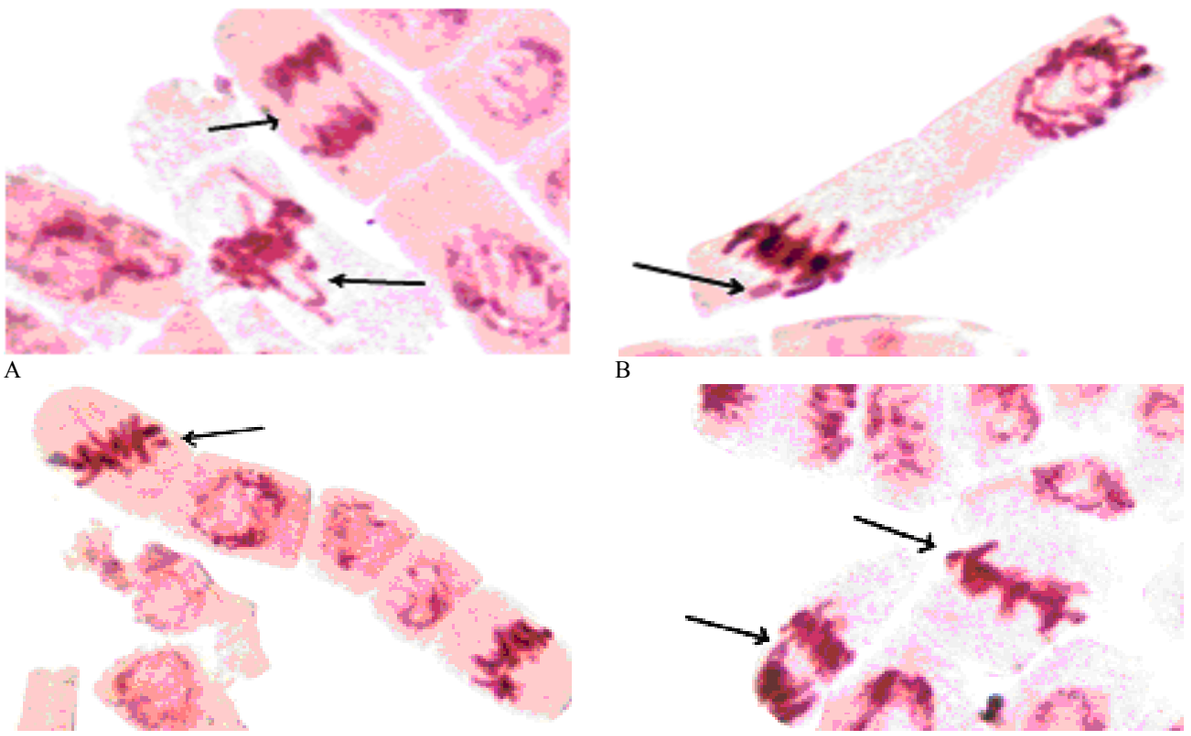


Fig. 7. Investigated chromosome abnormality (4 hour) D: chromosome breaking, A, D: chromosome dispersions, C: chromosomal adherence, D: fish bones chromosome adherence.



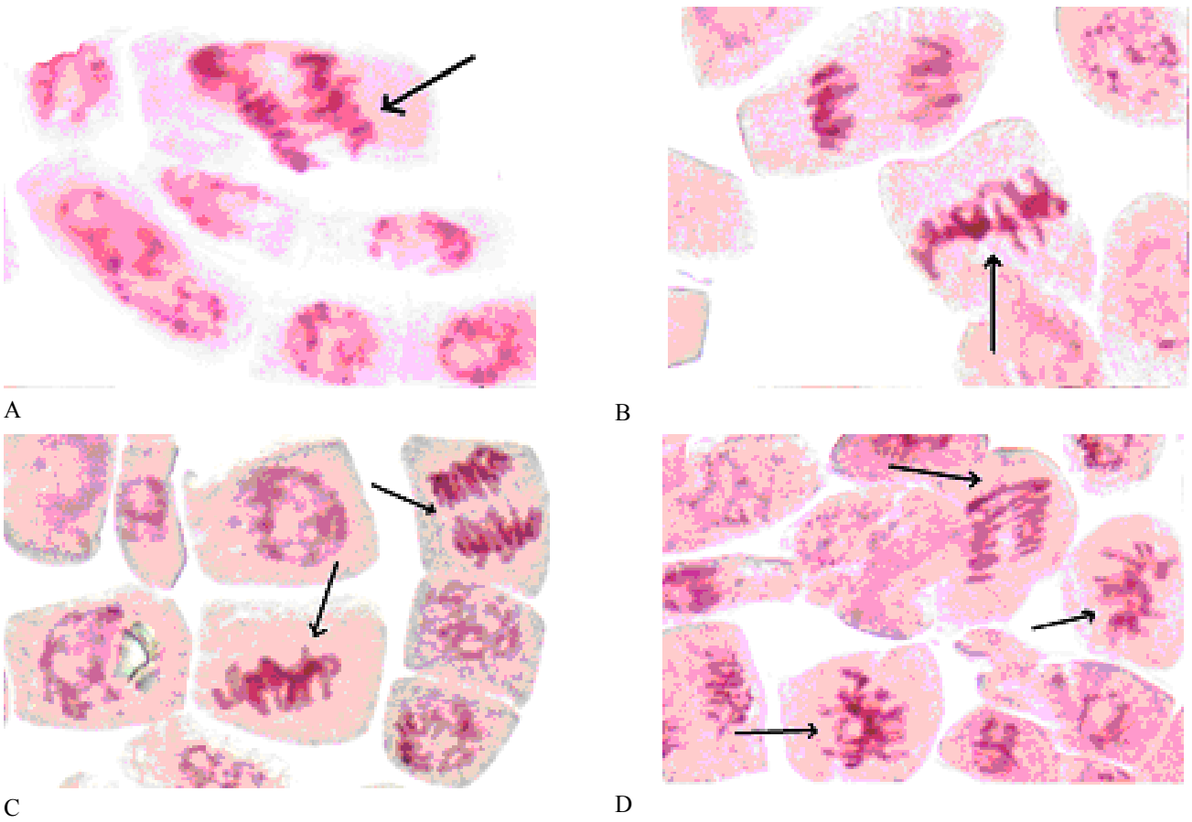
A B C D

Fig. 8. Investigated chromosome abnormality (8 hour) A, D: chromosome dispersions, D: chromosome breaking, A, B: bridge chromosome, C: fish bones chromosome adherence.



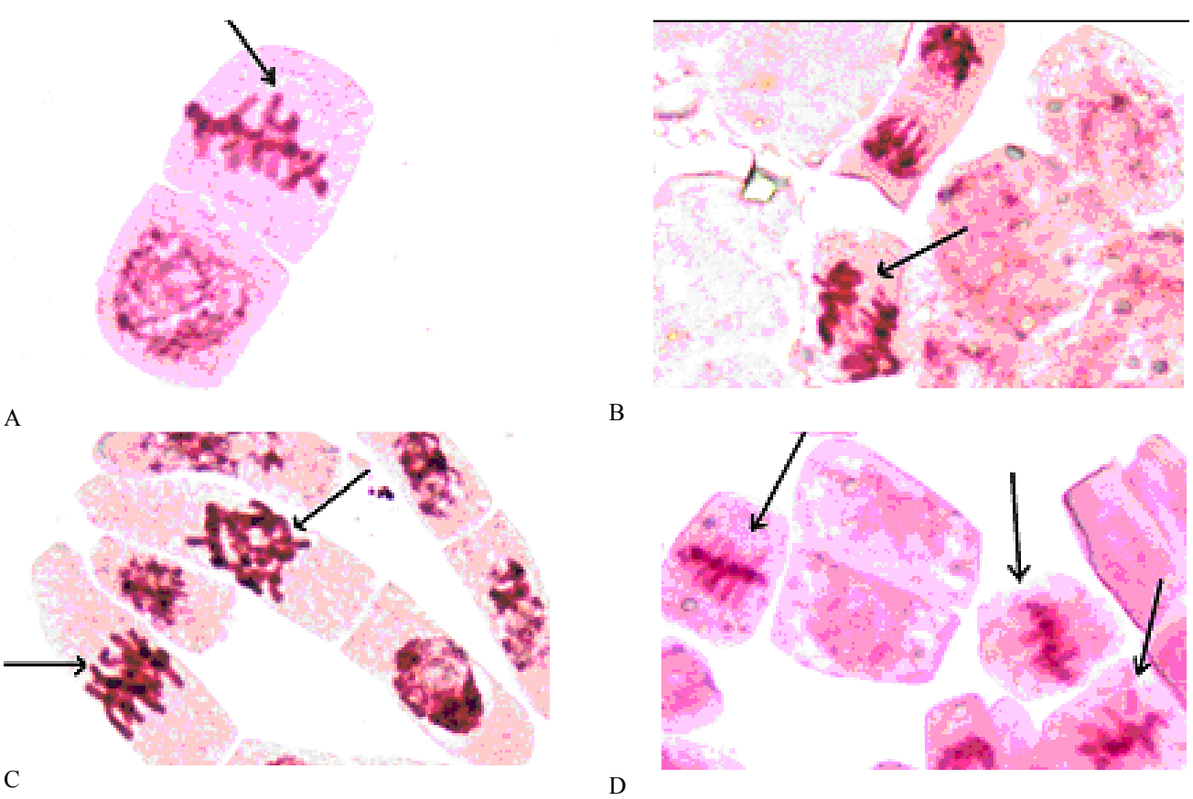
A B C D

Fig. 9. Investigated chromosome abnormality (12 hour) A: chromosome dispersions, A, D: bridge chromosome, B, C: chromosome breaking, D: fish bones chromosome adherence.



A B C D

Fig. 10. Investigated chromosome abnormality (16 hour) C, D: chromosome dispersions, D: chromosomal adherence, A, C, D: bridge chromosome, B: fish bones chromosome adherence.



A B C D

Fig. 11. Investigated chromosome abnormality (20 hour) D: chromosome breaking, A, C: chromosome dispersions, B: bridge chromosome, D: fish bones chromosome adherence, A: chromosomal adherence.

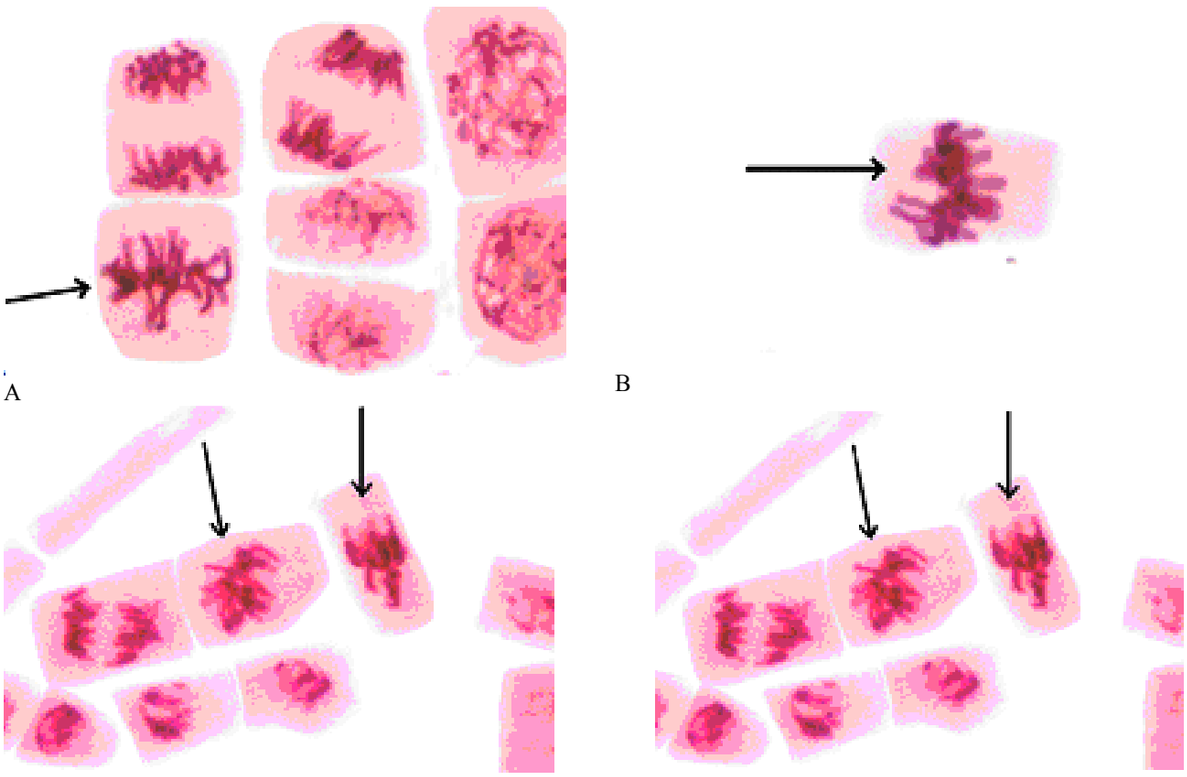


Fig. 12. Investigated chromosome abnormality (24 hour) A, C: chromosome dispersions, B: chromosomal adherence, D: bridge chromosome, C: chromosome shrinking.

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