










Effects of Radioiodine (I^{131}) on Mitotic Chromosomes of Root Meristem Cells of *Vicia faba* L. and Numerical Evaluation

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ABSTRACT: In this study Cytotoxic effects of radioiodine (I^{131}) which is a radionuclide used for the diagnosis and treatment of thyroid cancers and hyperthyroidism on mitotic chromosomes of root tips meristematic cells of fava bean [*Vicia faba* L. (Fabaceae)] were investigated. Seeds treated with radioiodine (I^{131}) for 24, 48, 96 hours and 16 days were germinated. The root tips were taken and prepared for microscopic studies. Radioiodine (I^{131}) caused chromosomal abnormalities as chromosome adherence, chromosome breaks, ring chromosome, chromatin bridge in *V. faba* L. root tip cells. The most common type of observed abnormalities was fish bone chromosome adherence, ring and C chromosome and chromosome dispersion. As a result of this study, it was determined that radioiodine (I^{131}) had a mitodepressive effect on mitosis and harmful effects on *V. faba* L. root tip cells. The data obtained were evaluated numerically and statistical analysis was performed.

Keywords: *Vicia faba* L., fava bean, chromosome abnormalities, radioiodine(I^{131}).

Radyoiodinin (I^{131}) *Vicia faba* L.'nin Kök Meristem Hücrelerinin Mitotik Kromozomları Üzerindeki Etkileri ve Sayısal Olarak Değerlendirilmesi

ÖZ: Bu çalışmada tiroid kanserlerinin ve hipertiroidizmin tanı ve tedavisinde kullanılan bir radyonüklid olan radyoiodinin (I^{131}), baklanın [*Vicia faba* L. (Fabaceae)] kök uçlarındaki meristematik hücrelerinin mitotik kromozomları üzerindeki sitotoksik etkileri incelendi. Tohumlar radyoiodin ile 24, 48, 96 saat ve 16 gün sürelerle muamele edildi ve çimlendirildi. Çimlenen kök uçları alındı ve mikroskopik çalışmalar için hazırlandı. Radyoiodine (I^{131}) *V. faba* L. kök ucu hücrelerinde kromozom yapışması, kromozom kopması, halka kromozom yapışması, köprü kromozom gibi kromozom anormalliklerine neden oldu. En çok görülen anormallikler balık kılıçığı kromozom yapışması, halka ve C kromozom, kromozom dağılmasıdır. Bu çalışmanın sonucu olarak, radyoiodinin mitoz üzerinde mitodepresif bir etkisi olduğu ve *V. faba* L. kök ucu hücreleri üzerinde zararlı etkileri olduğu tespit edildi. Elde edilen veriler sayısal olarak değerlendirilip istatistiksel analizleri yapıldı.

Anahtar Kelimeler: *Vicia faba* L., bakla, kromozom anormallikleri, radyoiodin (I^{131}).

INTRODUCTION

Radioiodine (I^{131}), an important radioisotope discovered by Glenn Seaborg and John Livingood in 1938 at the University of California, Berkeley, has a half-life of eight days (Valery, 2006). Radioiodine (I^{131}) scattered around the 1950s atomic bomb tests and nuclear disasters such as the Chernobyl and Fukushima nuclear catastrophes have made significant environmental health threats. Radioiodine (I^{131}) is formed by uranium and plutonium fission reactions. The fission resulting from these reactions constitutes about 3% of the products. Radioiodine (I^{131}) beta decay and causes death and mutations in affected cells and nearby cells. Therefore, high isotope doses are sometimes less dangerous than low doses. Because the radiation emitted by the radioiodine (I^{131}) can be transformed into cancerous cells tend to destroy thyroid tissues (Rivkees *et al.*, 1998). Cancers are caused by residual tissue radiation damage caused by radioiodine (I^{131}) and this may occur after years of exposure. It is assumed that the main cause of increased thyroid cancers after nuclear contamination is not caused by medical use, but by random spreading of radioiodine (I^{131}). (Simon *et al.*, 2006). According to the American Thyroid Association (ATA) 2015 guidelines, 1100MBq low dose radioiodine should be preferred in the treatment of thyroid cancer (Haugen *et al.*, 2016).

Iodine in foods, when these foods are consumed, is absorbed by the consumer's body and is usually accumulated in the thyroid glands. Because these glands need iodine for the do it's functioning. When high level radioiodine (I^{131}) present in the environment, it can be absorbed by foods, and pass on to the thyroid glands of people who eat these foods. When it decays, it can damage to the thyroid cells. The first risk of high-dose radioiodine (I^{131}) is the possibility of developing thyroid cancer later in life. There are some risks as non-cancerous growths and thyroiditis (Rivkees *et al.*, 1998). In addition, the medical use of radioiodine (I^{131}) is also used as a radiation detector in airports and the oil industry (Rao, 2006).

The main aim of this study is to determine chromosomal abnormalities occurring in on root tip cells of *V. faba* L. exposed to radioiodine(I^{131}) in different time periods.

MATERIAL and METHODS

Seeds of *V. faba* were kept in radioiodine (I^{131}) 24, 48, 96 hours and 16 days and germinated in petri dishes. The germinated root tips were cut and put in fixative, and stocked in 70% ethyl alcohol. The root tips were prepared according to the Feulgen method (Darlington and La Cour, 1976) and the preparats were used to count mitotic cells numbers, and to determinate chromosomal abnormalities.

The preparation was prepared using 5 root tips for each radioiodine application. Twenty image fields were chosen on these preparations for cytogenetic examination and chromosome abnormalities were detected. Approximately 600 cells were evaluated for each application. The photographs were taken using these preparations with a motorized Leica DM 3000 microscope (Leica Microsystems, Wetzlar, Germany). Chromosome abnormalities detected in the study have been coded as A, B, C, D, E, F, G and H. times of treatment have been coded as 1-4 (Table 2-4). Statistical analyses have been performed by using Analysis of Variance, Regression analysis and Pearson Correlation tests. The differences have been evaluated with the same tests. The reason for the application of this statistical method is to see if there is a difference between the groups on the variables studied. Thus, we have tried to prove and evaluate the results obtained from laboratory studies numerically. Statistical analyses have been performed using MINITAB software package.

RESULTS

In this study, the effect of radioiodine (I^{131}) on mitotic cell division in the root tip of *V. faba* was investigated. The number of mitotic division in the cells at the root tips of the seeds kept in the

standard radioiodine (I^{131}) solution in different time periods was increased compared to the control group. Mitotic activity and chromosomal abnormalities were most frequently observed in root tip cells that were maintained for 96 hours in radioiodine (I^{131}).

It was been observed that radioiodine (I^{131}) caused different chromosomal abnormalities as fish bone chromosome, chromosome dispersion, chromosome adherence, chromosome breaking, bridge chromosome, chromosome shrinking, ring chromosome at different phase of mitotic division and different time periods (Figure 2 and 3).

The most seen abnormality was fish bones chromosome. Fish bones chromosome, bridge chromosome and ring chromosome were observed all treatment hours except to 48-hour treatment time. All chromosomal abnormalities were identified as the highest level at 16-day treatment, while the lowest chromosomal abnormalities were identified at 24 hours. No mitotic activity and abnormalities were seen at 48-hour of treatment. It was been observed that the tips of the roots were completely decayed at radioiodine (I^{131}) standard

48 hours treatment (Figure 1: A,B,C,D; Figure 2; Figure 3; Table 1 and 2).

The statistical analyses of the results are shown in Tables 1, 2, 3, 4.

According to the statistical results derived, there is a considerable positive relation between the increase time of treatment and the mitotic index (%) except 48h. On the other hand, there are positive relation between the time of treatment and the chromosome abnormality (%) except 48h. (Table 3 and 4).

Types and proportions of chromosomal abnormalities and the percentage of total abnormalities produced by the different treatments of radioiodine (I^{131}) in *V. faba* root are given in Figure 4.

According to Table 3 and 4 based on the Pearson's correlation and analysis of variance method (Correlation), there are important correlations among (A-E; B-C, G, H; E-F, G; D-E; E-F; F-G, H) the investigated chromosome abnormalities at levels of 0.01 and 0.05 (Table 3 and 4).

Table 1. The mitotic index and total chromosome abnormalities in the root tip cells of *V. faba*.
Çizelge 1. *V. faba* kök ucu hücrelerinde mitotik indeks ve toplam kromozom anormallikleri yüzdesi.

	Time (Süre)				
	Control	24h	48h	96h	16d
Mitotic index (%) \pm SD	11.65 \pm 5.3	13.31 \pm 3.3	12.05 \pm 4.6	14.48 \pm 5.0	15.65 \pm 6.6
Total abnormalities (%) = (Abnormal cell number / Total cell number) X100	0.00	1.67	0.00	2.83	4.07
The number of different chromosome abnormalities	0.00	10	0.00	17	24

§ Mitotic index (Mitotik indeks); Total abnormalities (Toplam anormallik); Abnormal cell number (Anormal hücre sayısı); Total cell number (Toplam hücre sayısı); The number of different chromosome abnormalities (Farklı kromozom anormallikleri sayısı). Control (Kontrol); S.D (Standart Deviation (Standart Sapma); h: Hour (Saat); d: Day (Gün).

Table 2. The radioiodine (I^{131}) induced chromosome abnormalities in the root tip cells of *V. faba*.
Çizelge 2. *V. faba* kök ucu hücrelerinde radyoyodinin (I^{131}) tetiklediği kromozom anormallikleri.

Chromosome abnormalities (%) Kromozom anormallikleri (%)		Time (Süre)			
		24h (1)	48h (2)	96h (3)	16d (4)
Fishbone chromosome	A	0.83	0.00	1.17	0.68
Chromosome breaking	B	0.33	0.00	0.00	0.68
Chromosome shrinkage	C	0.00	0.00	0.33	0.17
C Chromosome	D	0.17	0.00	0.17	0.00
Chromosome adherence	E	0.00	0.00	0.00	0.17
Chromosome dispersion	F	0.00	0.00	0.83	1.69
Ring chromosome	G	0.17	0.00	0.17	0.50
Bridge chromosome	H	0.17	0.00	0.17	0.17

Abbreviations: A-H: Codes of chromosome abnormalities; 1-4: Codes of treatment times: (24h): 1, (48h): 2, (96h): 3, (16d): 4.

Table 3. Pearson's correlation based on chromosome abnormalities.

Tablo 3. Kromozom anormalliklerine dayalı Pearson korelasyonu.

Chromosome abnormalities (Kromozom anormallikleri)							
	A	B	C	D	E	F	G
B	0.130						
	0.870						
C	0.699	0.034*					
	0.301	0.916					
D	0.801	0.270	0.306				
	0.199	0.730	0.694				
E	0.099	0.850	0.285	0.484			
	0.901	0.150	0.715	0.516			
F	0.361	0.670	0.619	0.267	0.929		
	0.639	0.330	0.381	0.733	0.021*		
G	0.361	0.912	0.326	0.194	0.946	0.906	
	0.639	0.048**	0.674	0.806	0.014**	0.044**	
H	0.888	0.568	0.497	0.567	0.440	0.565	0.698
	0.112	0.432	0.503	0.433	0.560	0.435	0.302

* Significant at the level of $P < 0.05$; ** Significant at the level of $P < 0.01$ (* $P < 0.05$ düzeyinde anlamlı; ** $P < 0.01$ düzeyinde anlamlı).
Abbreviations: A-I : Codes of chromosome abnormalities (Kısaltmalar: A-I: Kromozom anormalliklerinin kodları).

Table 4. Correlation between 8 investigated chromosome abnormalities (Analysis of Variance).

Tablo 4. Belirlenen 8 kromozom anormalliğinin varyans analizi ile karşılaştırılması.

Abnormalities / Anormallikler	MS	F-value	Probability	Significance
A-B	0.340	2.02	0.205	NS
A-C	0.583	4.49	0.078	NS
A-E	0.763	6.28	0.046	*
B-C	0.673	5.49	0.050	*
B-H	0.325	0.61	0.460	NS
D-E	0.281	0.40	0.549	NS
E-F	0.763	6.28	0.046	*
E-G	8.583	7.43	0.020	**
F-G	0.815	4.40	0.050	*
F-H	0.510	1.58	0.256	NS

MS: Mean Square * $P < 0.05$ ** $P < 0.01$; A-H: Codes abnormalities; NS: Not Significant.

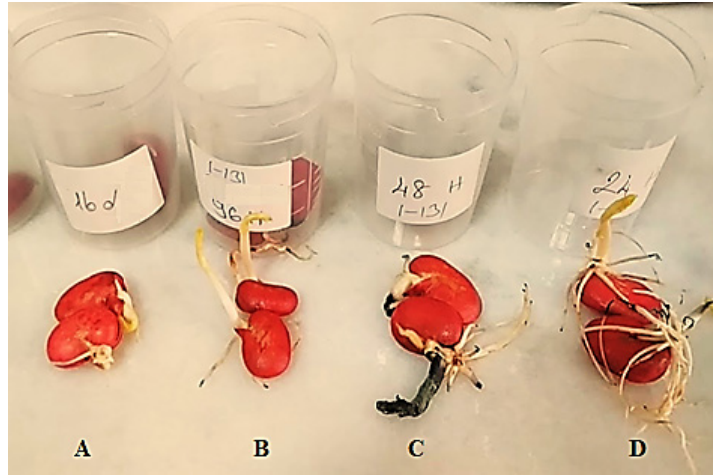


Figure 1. General views of *Vicia faba* L. seeds treated with radioiodine (I^{131}) at different durations [A (16 day), B (96 hour), C (48 hour), D (24 hour)].

Şekil 1. Farklı sürelerde radyoyodinin (I^{131}) uygulanan *Vicia faba* L. tohumlarının genel görünüşleri [A (16 gün), B (96 saat), C (48 saat), D (24 saat)].

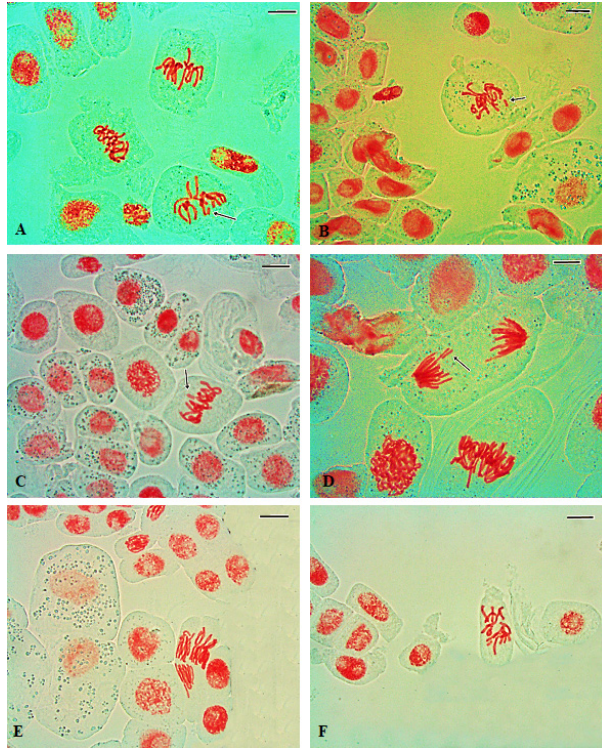


Figure 2. Chromosomal abnormalities produced by radioiodine (I^{131}) in *V. faba*. Chromosome breaking (A, B, D), Chromosome adherence (C, D, E), Chromosome dispersion (E), Ring chromosome (C, F) (Scala Bar 10 μ m).

Şekil 2. *V. faba*'da radyoyodinin (I^{131}) neden olduğu kromozomal anomallikler. Kromozom kırılması (A, B, D), Kromozom yapışması (C, D, E), Kromozom dağılımı (E), Halka kromozomu (C, F) (Ölçek 10 μ m).

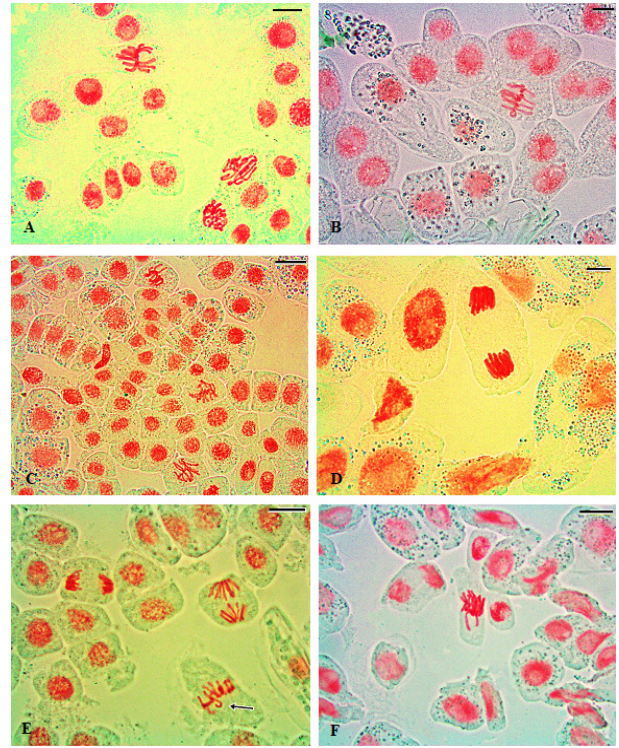


Figure 3. Chromosomal abnormalities produced by radioiodine (I^{131}) in *V. faba*. Fish Bone Chromosome (A, B, C, E), Chromosome adherence (A, F), Chromosome dispersion (A, F), Chromosome shrinkage (D), C Chromosome (A, E, F), Ring chromosome (B), Bridge Chromosome (E). (Scala Bar 10 μ m)

Şekil 3. Radyoyodinin (I^{131}) *V. faba*'da neden olduğu kromozomal anomallikler. Balık kılıçığı kromozomu (A, B, C, E), Kromozom yapışması (A, F), Kromozom dağılımı (A, F), Kromozom büzülmesi (D), C Kromozomu (A, E, F), Halka kromozomu (B), Köprü Kromozomu (E). (Ölçek 10 μ m).

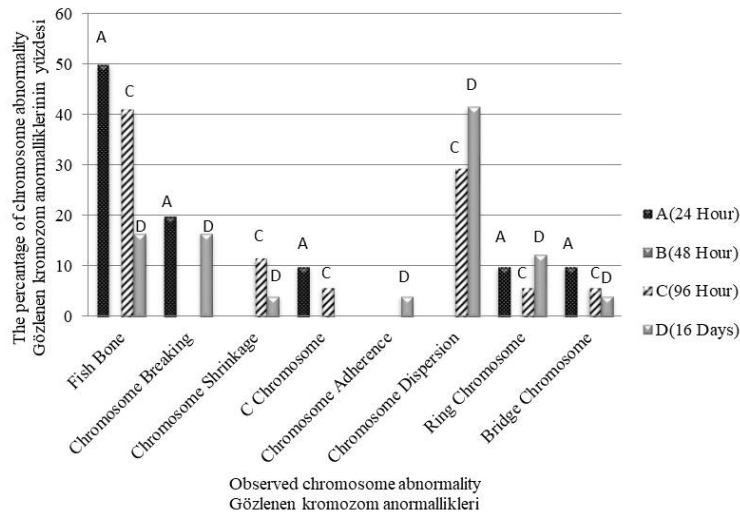


Figure 4. Types and proportions of chromosomal abnormalities and the percentage of total abnormalities produced by the different treatments of radioiodine (I^{131}) in *V. faba* root.

Şekil 4. *V. faba* kökünde farklı sürelerle radioiodine (I^{131}) uygulanmasıyla ortaya çıkan kromozom anomallik tipleri ve oranları.

DISSCUSSION

In present study, cytogenetic effects of radioiodine (I^{131}) on meristem cells of *V. faba* root tips was investigated. *V. faba* seeds were kept in radioiodine (I^{131}) for increasing time period as control during, 24, 48, 96 hours.

It was determined that radioiodine (I^{131}) was caused to some chromosomal abnormality at the *V. faba* as chromosome shrinking, fish bones chromosome adherence, ring chromosome, chromosomal adherence, chromosome dispersions, bridge chromosome, chromosome breaking (Figure 2,3,4). Similarly, it has been reported that copper chloride and uranium have provoked to some chromosomal abnormality on *V. faba* and *Vicia hirsute* root tip cells. It has been determined that uranium affects the frequency of mitotic cell division and causes some chromosomal abnormality on *V. faba* and *V. hirsute* cells at different time periods (Özdemir *et al.*, 2008; İnceer *et al.*, 2003). In another study, the researchers observed that cell division was decreased and caused several mitotic anomalies such as multipolar anaphases, chromosome bridges, c mitosis and lagging chromosomes on root tip cells of lentil (*Lens culinaris* Medik.) at increasing

concentrations of lead ($PbCl_2$) (Kıran and Sahin, 2005).

The researchers found similar results for different radioactive elements in literature. In their study, high concentrations of uranium significantly stimulate seedling growth. The results show that low uranium concentrations increased nuclease (RNase) activity while higher uranium concentrations inhibited. As uranium concentrations increased, the inhibitory effect increased. So, uranium has effects on the metabolic activity of nuclease (Yi *et al.*, 2007).

The effect of the cadmium chloride in the *V. faba* root tips was studied and evaluated in relation to the mitotic cell division and chromosomal abnormalities rate. Seeds germinated for 48 hours in different concentrations of cadmium chloride. It was showed that different chromosome abnormalities such as lagging chromosome, polyploidy, chromosomal bridge, multipolarity and micronuclei. The mitotic division rate was decreased with increasing cadmium concentration (Parween *et al.*, 2011).

Radioactive materials can transport from soil to autotrophic organism and then can reach heterotrophic organisms as human. There is not

enough study about the role of transition metals in soil and organisms (Kasianenko and Kulieva, 2002). In the literature, some researchers determined that cytogenetic effect of copper, zinc, lead and chrome on root tip cells of *Allium cepa* (Arambasic *et al.*, 1995). Leonard *et al.*, (1983) investigated that how mercury affects spindle fibers during the mitotic division of root tip cells of *V. faba* and *A. cepa*. This result shows that the application time of radioiodine (I^{131}) on the seeds for mitotic division is important (Figure 1, 2, 3; Table 1, 2).

According to the analysis of variance, regression analysis and Pearson Correlation Tests results, different application time of radioiodine (I^{131}) on

the seeds was caused different chromosome abnormalities (Table 3, 4).

It has been found that there have been statistically important differences at levels of 0.01P among to Chromosome adherence (E), Chromosome dispersion (F) and Ring chromosome (G) (Table 3, 4). We can say that these chromosomal abnormalities are interrelated and trigger each other.

Radioiodine (I^{131}) is used for the treatment of some types of thyroid cancer and thyrotoxicosis. In this study, we intended to determine cytogenetic effect of radioiodine (I^{131}) on the root tip cells of *V. faba* whose seeds are widely consumed by people as food.

REFERENCES

- Arambasic, M. B., S. Bjelic, and G. V. S. Simic. 1995. Acute toxicity of heavy metals (Copper, Lead, Zinc), phenol and sodium on *Allium cepa* L., *Lepidium sativum* L., *Daphnia magna* St. comparative investigations and the practical applications. *Water Research* 29 (2): 497-503.
- Darlington, C. D., and L. F. La Cour. 1976. *The Handling of Chromosomes*. 6th ed. Allen and Unwin, London.
- Haugen, B. R., E. K. Alexander, K. C. Bible, G. M. Doherty, S. J. Mandel, Y. E. Nikiforov, F. Pacini, G. W. Randolph, A. M. Sawka, M. Schlumberger, K. G. Schuff, S. I. Sherman, J. A. Sosa, D. L. Steward, R. M. Tuttle, and L. Wartofsky. 2016. American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid* 26 (1): 1-133.
- İnceer, H., S. Ayaz, O. Beyazoğlu, and E. Şentürk. 2003. Cytogenetic effects of copper chloride on the root tip cells of *Helianthus annuus* L. *Turkish Journal of Biology* 27 (1): 43-46.
- Kasianenko, A. A., and G. A. Kulieva. 2002. Ecological consequences of depleted uranium use in ammunition. *Bulletin of Russian Peoples' Friendship University. Series Ecology and Life Safety-Moscow*, Publishing House of RPFU 6: 90-99.
- Kıran, Y., and A. Sahin. 2005. The effects of the lead on the seed germination, root growth and root tip cell mitotic divisions of *Lens culinaris* Medik. *Gazi Univ. Journal of Science* 18 (1): 17-25.
- Leonard, A., P. Jacquet, and R. Lauverys. 1983. Mutagenicity and teratogenicity of mercury compounds. *Mutat Res.* 114 (1): 1-8.
- Özdemir, C., F. S. Erees, and S. Çam. 2008. Cytogenetic effects of uranium on root tip cells of *Vicia faba* L. *Botanica Lithuanica* 14 (3): 155-158.
- Parween, T., S. Jan, Mahmooduzzafar, M. P. Sharma, A. Mujib, and T. Fatma. 2011. Genotoxic impact of cadmium on root meristem of *Vicia faba* L. *Russian Agricultural Sciences* 37 (2): 115-119. <https://doi.org/10.3103/S1068367411020248>.
- Rivkees, S. A., C. Sklar, and M. Freemark. 1998. The Management of graves' disease in children, with special emphasis on radioiodine treatment. *Journal of Clinical Endocrinology and Metabolism*. 83 (11): 3767-3776.
- Simon, S., A. Bouville, and C. Land. 2006. Fallout from nuclear weapons tests and cancer risks. *American Scientist* 94 (1): 48-57. <https://doi.org/10.1511/2006.57.48>.
- Rao, S. M. 2006. Radioisotopes of hydrological interest. *In: Practical isotope hydrology*. New Delhi: New India Publishing Agency 12-13.
- Valery, M. D. 2006. Biogenic iodine and iodine-containing metabolites. *NPC Natural Product Communications* 1 (2): 139-175.
- Yi, S., R. L. Wang, T. Feng, Y-C. Xiang, and H. Zhou. 2007. Effects of uranium tailings lixivium on early growth of *Vicia faba*. *Journal of Hunan University of Science and Technology (Natural Science Edition)* 4: 113-116.