NUKLEONIKA 2012;57(3):421–426

ORIGINAL PAPER

Search of radiation hormesis in plants: irradiation of the cress (*Lepidium sativum* L.)

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Abstract. The paper is composed of two parts: a review of a group of experiments among irradiated plants and own search for radiation hormesis in a single experiment. In the first part the Bayesian analysis of the hormetic-like data published so far shows that the NOAEL (no observed adverse effect level) point, above which adverse effects appear, may be located between 30 and 100 Gy. In the second part the influence of low doses of ionizing radiation was tested on the particularly fast growing plant, namely the cress (*Lepidium sativum* L.). Two experimental scenarios were used: in the first one the cress was irradiated during the growth (maximal dose 2.3 Gy), while in the second scenario dry seeds were irradiated (maximal dose 100 Gy). The experiment indicates that the NOAEL point lies above 100 Gy and statistically insignificant hormetic effect can be seen between 0.1 and 14 Gy. No linear reaction is observed in the full range of doses.

Key words: cress • hormesis • ionizing radiation • *Lepidium sativum* L. • low dose

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Received: 1 February 2012
Accepted: 26 March 2012

Introduction

The influence of ionizing radiation on plants were often studied in recent decades. A review of such investigations can be found, e.g. in [2, 18]. The cited papers show examples of radiation hormesis found in irradiated plants.

The hormetic effects in plants are rather small, they usually do not exceed 10% of the measured parameter and are sometimes neither reproducible nor independently confirmed [18]. The measured parameters are various: one can test the height, weight, growth rate, flowering, sprouting, etc. Also one can study how different species of plants grow during days, weeks, months or even years. During such studies, the radiation hormesis is often detected [22].

In most cases the hormetic effect is observed after irradiation of seeds, so the storage time after seed irradiation have to be minimized to gain the greatest response [22]. A long delay between irradiation and planting can cause a significant decrease of the response – the hormetic effect can be potentially seen when this delay does not exceed 150 days [1, 23].

As a rule, high doses of ionizing radiation are detrimental to plants. However, one should note that the term "high dose" is much different for plants than for humans and animals, which follows from subcellular structure of these organisms. Great difference between harmful or lethal dose for plants and animals is quite obvious if one notes that plants have cell walls and totipotential organization of cells that provide great regenerational ability. Also, in the case of plants, the lack of possibility to move gave a reason to evolutional development of more effective system of repair of various cellular damages due also to the ionizing radiation [13]. Usually, the analogical doses to plants are at least one order of magnitude greater than those considered for animals [18]. In the low dose region the situation is much more complicated. Mostly hormetic effect is found, whereas some of the studies show either no effect or indicate simple threshold reaction [4, 5, 11].

In the present paper a group of popular studies, showing hormesis in plants, are re-analyzed. If hormesis appears, the reaction of plants to the ionizing radiation should exhibit a NOAEL (no observed adverse effect level) point [3]. The data obtained so far as well as the new data obtained after irradiating quickly-growing plant – the cress (*Lepidium sativum*) are analyzed. The choice of cress stemmed from the hope that in this quickly growing plant one could find some hints of hormesis in the low-dose region.

Radiation hormesis in plants

The data

Figure 1 presents the data from various studies [2, 8, 18–20], including the present paper (section 'Irradiation of the cress'). The vertical axis represents the effect of irradiation – usually height but also weight, growth rate, flowering and sprouting in some cases, as a relative ratio to the control group. All results are presented by a comparison with non-irradiated control group (using the so-called relative ratio standard): when the effect is positive (i.e. hormetic), the result is higher than 1, when the effect is detrimental the result is lower than 1 (e.g. result 1.1 means the hormetic effect on a level of 10%). If the situation in control and exposed groups is the same, the result (relative ratio) equals 1. The horizontal axis represents the dose of ionizing radiation to a plant, usually to its dry seeds. Figure 2 contains the same data, but in a logarithmic scale, to highlight the results in the lowest doses. A comparison of Figs. 1 and 2 is of certain interest because representation of the data in logarithmic scale is quite common.

A quick view to Fig. 1 shows that the data can be subdivided into three parts: below about 30 Gy almost all

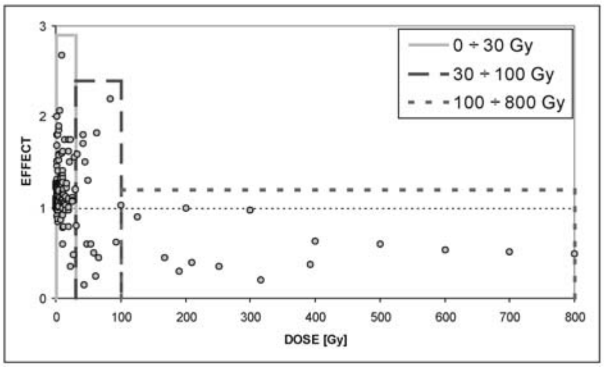

Fig. 1. Collection of the data. Broken lines show distinctions discussed in the text. See also Fig. 2. with the same data in the logarithmic scale.

of them show hormetic reaction to the dose of ionizing radiation. Above about 100 Gy, the detrimental effects growing perhaps linearly with dose are observed. Unfortunately, the experimental uncertainty of points were not published – the authors of cited papers do not usually show this important characteristic of the data quality. In spite of this difficulty, the use of Bayesian methods of statistical analysis was considered. In order to do that it was assumed that all experimental points are characterized by the same uncertainty, set arbitrarily to unity.

The analysis

The Bayesian analysis is one of the most popular algorithms in the data analysis when the data are not certain or are incomplete. The details (including calculations) can be found in a textbook by [25], and also in [6, 7]. As shown in the latter papers, the Bayesian analysis is especially useful when the data contain outliers or have a large scatter of experimental points, e.g. as seen in Figs. 1 and 2. By accepting the fact that the uncertainties given by the authors of original papers may be wrong, the algorithm tests how well the points can be described by assumed functions if the uncertainties could be questioned and subdued to certain probability distribution. In the present paper we followed the prescription given in [6].

The first goal was to answer an important question: is there a strict dose above which detrimental effects of ionizing radiation appear? This point in all hormetic models is called NOAEL [3]. This can also be the value of a threshold if instead of hormetic model one considers the model of threshold reaction. However, one has to be aware that still the most frequently used paradigm is a linear growth of the risk with dose, which definitely excludes both possibilities mentioned above and says that even a minute dose of ionizing radiation induces adverse effects.

Three simplest mathematical models were tested according to the data presented in Figs. 1 and 2: the constant (dose-independent), linear and parabolic (quadratic) models. All of them were fitted to all experimental data using classical (maximum likelihood) and Bayesian algorithms [6, 7]. It is important to recall that in the latter approach, all the points are at the start assumed to have the same weight, because in the original papers the authors missed the uncertainty values. This quite confusing situation is met in most of the papers reporting strictly biological results.

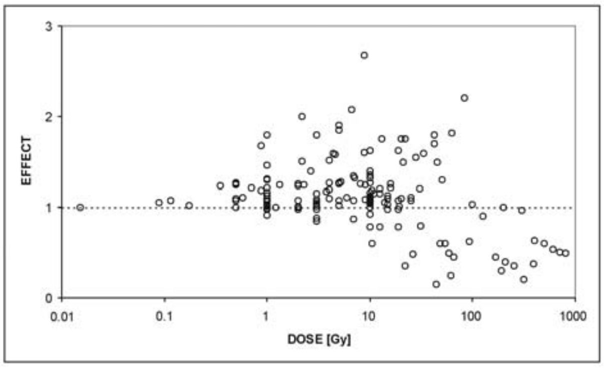

Fig. 2. Data points from the Fig. 1 in the logarithmic scale.

When the values of models' fitting parameters are found, the Bayesian analysis can also test the relative plausibility of the models. This test is called model selection algorithm [7, 25] and is very useful when one has to compare the relative plausibility of models. In a more conventional approach one can also be directed by the value of the well-known misfit function, χ^2.

Results

The first look into the data depicted in Fig. 1 shows that only above the dose $D = 100$ Gy one observes mainly detrimental effects of ionizing radiation. Below this dose, the data can be described by almost any function. However, in the simplest approach one can check the case of dose-independent (constant) reaction, $T(D) = a$, and the linear reaction, i.e. described by $T(D) = aD + b$, where a and b are parameters to be fitted. In the dose--independent model one gets the value $T(D) = 1.18 \pm 0.13$ with $\chi^2 = 18.5$, while in the linear model one obtains $a = -0.002 \pm 0.009$, $b = 1.21 \pm 0.15$ with $\chi^2 = 18.2$. The value of NOAEL resulting from this parameters is 105 Gy, however with large uncertainty of about ±500 Gy, which clearly indicates that within this range of doses precise estimation of NOAEL is not possible. It is easily seen that this high uncertainty is mainly due to very low precision of the estimation of the slope parameter, a. The absolute values of χ^2 obtained in both cases are less relevant, however, relative values matter. From the quoted values, it follows that the linear model works not better than the model with the reaction independent of the dose, and the slope a obtained is practically zero within the calculated accuracy. What is truly important is that one consistently obtains hormetic values below $D = 100$ Gy. Such a result, however, has to be treated with a certain caution as one cannot expect any effect at $D = 0$, i.e. one should have $T(D) = 1$. The values higher than 1, that indicate hormetic effect, represent weighted average of the results, in which the positive values apparently dominate.

The closer look at the data shows that 135 out of all 166 data lie in the region below 30 Gy (Figs. 1 and 2). Most of these points are having values larger than 1, and just these points are weighting most in the analysis. Without going to the more sophisticated Bayesian analysis, it is easy to check (by means of conventional maximum likelihood analysis) that the most sensible conclusion arising from these data is that in this region of doses one sees, on the average, the effect on the level of 1.2, in full agreement with the Bayesian analysis.

In order to have zero reaction at $D = 0$ one could fit a parabola, which would correspond to the hormetic curve [15]. The mathematical formula for such a parabola is $T(D) = aD^2 + bD + 1$. However, the overall agreement with the data (as judged by the value of χ^2) is not better than that obtained so far, while the plausibility of this model is apparently lower than of the model in which the effect is dose-independent. Also the values of parameter uncertainties are high, so in fact, the quadratic model has no statistical power irrespective of the statistical method used in the analysis.

The data above 100 Gy are mostly exhibiting detrimental effects, increasing with increasing dose. What happens in the range of 30 to 100 Gy is difficult to say. The number of points in this range is not large and the number of hormetic and detrimental results are quite the same. Therefore, one say that the most likely value of NOAEL must lie within this range.

Irradiation of the cress

To supplement the results published so far, a simple experiment, in which the quickly-growing plant like the cress (*Lepidium sativum* L.) was irradiated, has been carried out.

Methods

The irradiation case of cress, to the best knowledge of the authors, was studied only once [16]. These studies, however, have been performed at rather high doses, above 200 Gy.

The experiment presented in this paper was thus divided into two stages:

– first stage, where the cress was growing in the gamma ionizing radiation field;
– second stage, where the dry seeds of cress were gamma irradiated, and sown after few days.

The experiment was carried out in 2010 in the National Centre for Nuclear Research (Otwock, Poland) and at the Institute of Biochemistry and Biophysics, Polish Academy of Sciences (Warsaw, Poland). All seeds of the cress used in the experiment came from the same cultivation and had the same serial number to keep the homogeneity and avoid any confounding factors.

First stage – growth in ionizing radiation field

The cress seeds were sown in a long linear tray. The radiation source was situated near one of the ends of the tray. The seeds were sown on wet wadding, which was watered once a day. First, the plants were growing during 11 days in natural light. Next, the experiment was repeated in the darkness to test the sensitivity of growth into the illumination of light.

The measurements (the length of stalks) were taken after 7 and 11 days.

Second stage – germination and growth of irradiated seeds

The second stage was divided into two experiments where 8 samples of seeds were used.

In the first experiment the sprouting force was measured (Fig. 3). The sprouting force is the percent of germinated seeds after 48 h when they were kept in a still wet small scale pan. The scale pan was put into a phytotron (bioclim cabin), where the temperature (20°C), humidity and light were constant.

In the second experiment the seeds were sown on vermiculite, also in the phytotron. The percent of germinated seeds and the total length of stalks and roots were measured after 7 days of their growth in the same conditions. The experiment was repeated on wet wadding in natural light, instead of vermiculite and the phytotron.

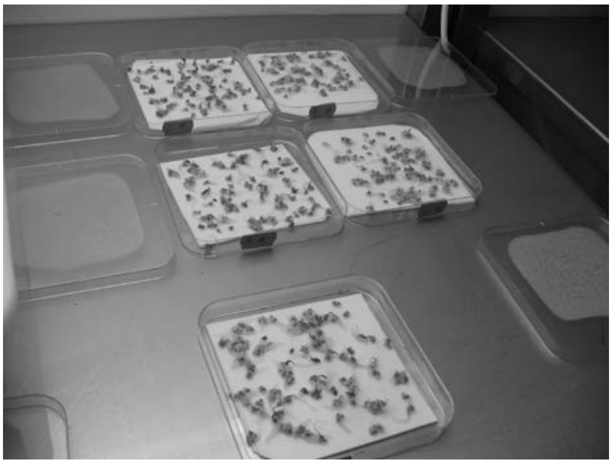

Fig. 3. The examplary picture from the experiment – the selected cress' sprouts after 48 h in a phytotron chamber.

Dosimetry

The gamma-ray source used while the cress was irradiated during the growth, was Ir-192 with on activity of 50 GBq. The value of doses were measured using photometric dosimeters type 2 of Kodak. Doses measured with these dosimeters were read out by an accredited laboratory. Uncertainty of their readings were estimated to be 22%. Because the irradiation time was long (up to 11 days), its uncertainty had little influence on the final accuracy of calculate doses.

Dry seeds were gamma irradiated with two different dose rates, using two different systems. Small doses (below 4 Gy) were measured with the same equipment as above. Higher doses were calculated from the known geometry of source-sample configuration and the source characteristics (also Ir-192). Cumulative dose uncertainties were estimated to be not higher than 20%. This estimation includes uncertainty of relatively short measuring time whose error could have greater impact on calculated dose than in the previous case.

All absorbed doses presented in this paper are cumulative ones, measured in grays (Gy). All results are shown with uncertainties of one standard deviation (SD) (68% CI, confidence intervals).

Results

In the first stage, where plants were irradiated during the growth, no dose-response was detected. Within the uncertainty of measurements, the height of plants was insensitive to the irradiation dose.

The relative growth (RG) of the cress in natural light after 7 days for maximally irradiated sample (1 Gy) was RG = 1.02 ± 0.11. After 11 days, the maximally irradiated sample (1.6 Gy) showed RG = 0.99 ± 0.23.

When the irradiation was carried out in the darkness, the RG after 7 days, for maximally irradiated sample (1.5 Gy), was RG = 0.90 ± 0.12 and after 11 days, the maximally irradiated sample (2.3 Gy) results RG = 1.03 ± 0.22.

Part of the results obtained in the second stage are presented in Fig. 4. Below 100 Gy, the percent of germinated seeds after 48 h (the sprouting force) was not

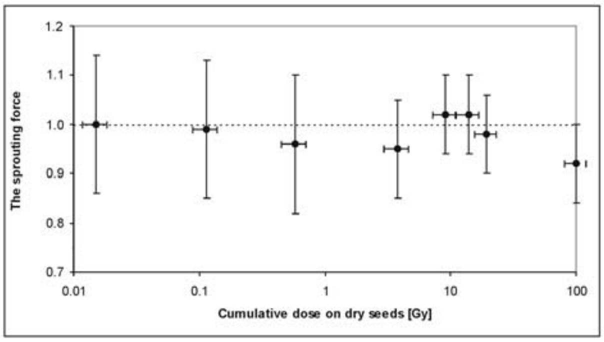

Fig. 4. The sprouting force of cress after 48 h as the relative number of germinated seeds. The point at 100 Gy (results 0.92 ± 0.08) shows potential threshold. The dotted line corresponds to the control group.

different from the one in the control group (no response to irradiation). The experiment indicated, however, an adverse effect above the threshold of about 100 Gy (possible NOAEL point).

The lengths of sprouts after 48 h were the same as in the controls.

In the case of the second experiment (seeds in vermiculite in the phytotron, also in natural light in the next attempt) the length of stalks and roots were the same as those of controls. Using the vermiculite in the phytotron, the percent of formed plants after 7 days showed probably statistically insignificant hormetic effect with a maximum at 3.8 Gy (where relative number of plants equals 1.17 ± 0.19) and the potential NOAEL above 100 Gy (Fig. 5). The length of stalks at 100 Gy was RG = 0.84 ± 0.23.

Discussion and conclusions

The experimental points collected in Figs. 1 and 2 enabled to make their inter-comparison and check whether any common NOAEL in irradiated plants exists [1, 2, 8, 18–24]. In fact, the data from Figs. 1 and 2 are different and show different results like the height, but also weight, growth rate, flowering and sprouting. The reason for collection and analysis of such vastly different data in one figure stems from the question whether any possible threshold point or hormetic effect among irradiated plants can be observed. Very similar approach could be found in the literature [7, 14].

Using all data together, two methods of statistical analysis were used: classical maximal likelihood and

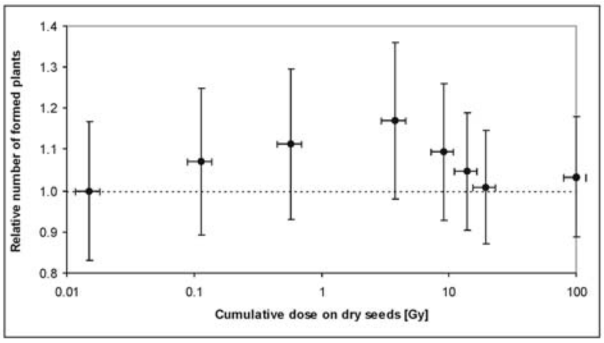

Fig. 5. The relative number of formed cress after 7 days. The dotted line corresponds to the control group.

the Bayesian one. However, in spite of the similarities, no practical advantage of using any statistical methods to the whole set of data was found because of a huge scatter of points. The data can be clearly divided into three groups: below 30 Gy, in the range of 30÷100 Gy, and above 100 Gy. The qualitative conclusions can be easily drawn up and one can say that neither linear nor parabolic models would describe well the whole group of data. Certainly, the linear model can be excluded because of the dominating positive reaction to low doses. At the doses below 30 Gy, one could estimate hormetic effect on the level of 20%, above 100 Gy one could claim detrimental effects linearly increasing with dose. In the intermediate region one could hardly say whether the effect is positive or negative. This is the reason why it is not possible to describe reliably the whole set of data by a single function.

In conclusion, one can say that the hormesis is typically observed below 30 Gy, and the NOAEL point must lie between about 30 and 100 Gy. This value for plants is at least one order of magnitude greater than similar values found among many species of animals [18].

The simple experiment with irradiation of the cress (*Lepidium sativum* L.) shows likely, although not statistically significant, NOAEL point between 100 Gy and 400 Gy (Fig. 6), which is in general agreement with the results obtained for other plants.

When the analyzed cress was grown in ionizing radiation field, no significant difference in plants germination or growth was observed. Applied doses were similar to the ones used in many other experiments which show hormesis [2, 18, 22]. One can find, however, similar experiments, for example performed for *Arabidopsis thaliana* [10], that show significant negative effect of irradation and no hormesis. One should note, that the scheme of the cited measurements was different: the plants were irradiated during the period of 15–25 days of their growth, and no irradiation of dry seeds was carried out. On the other hand, one cannot exclude that *Arabidopsis thaliana* and *Lepidium sativum* respond differently to radiation, because for a given species and cultivar, seeds and plants at various stages of development considerably differ in radiosensitivity [9]. More than that the response can be different "even inside the same species, different cultivars can assume different behaviours after exposure to the same conditions" [17].

The paper by Majeed *et al*. [16] brings the data on dry seeds of cress irradiated with doses larger than 200 Gy. In accordance with these data, the percent of survived plants decreased with dose. The combined results based on the presented data and the data from [16] are shown in Fig. 6. Results of the present studies (see also Fig. 5) suggest a possibility of hormetic effect. Majeed and collaborators [16] showed also that the length of stalks and roots decreased with dose above 200 Gy. According to Kurimoto *et al*. [10], the proper criteria of radiation effect would be the plant height measured together with stem mass, leaf mass, total leaf area, and above-ground biomass. Photosynthesis and respiration rates also are often used for estimation of the radiation effect. In contrast, germination of irradiated seeds as a measure of the radiation effect is less controversial and acceptable. On the whole, the hormetic effect is not easy to identify and this is also the case with the cress in the reported experiments.

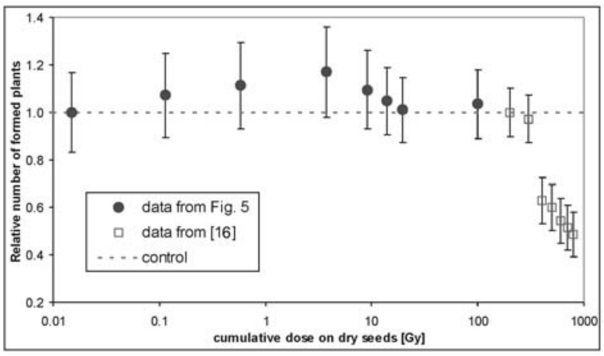

Fig. 6. The extension of Fig. 5 containing data above 100 Gy from [16]. The dotted line corresponds to the control group.

The main three goals of the presented simple experiment were: a) to compare the response of the cress to irradiation and check whether its potential hormetic reaction falls into the hormesis area observed on other plants, b) to find the dose-effect relationship at low doses for the cress and c) to supplement the experiment by Majeed and colleagues [16].

The adaptive response effect (connected or often identified with radiation hormesis) is well known to help the plant growth in higher than usual doses of ionizing radiation [4, 12, 15]. It follows from the presented experiment on the cress that possible, although statistically insignificant, hormetic effect between 0.1 and 14 Gy is observed. The effect of radiation hormesis (strictly connected with adaptive respose), reported many times [2, 11, 18, 22], turned out to be rather small for cress growth. The threshold model may, however, describe the presented and Majeed *et al*. data [16] equally well.

Acknowledgment. The authors wish to express their gratitude to Prof. L. Dobrzyński and to J. Rauk, M. Witas and T. Ostrowski from the National Centre for Nuclear Research (NCBJ, Otwock, Poland) for all help, as well as to the Institute of Biochemistry and Biophysics, Polish Academy of Sciences (Warsaw, Poland) for letting us the use of phytotron chambers. The research was supported by European Union as a part of European Social Fund (PO KL 2007-2013, priority VIII, subaction 8.2.2 Regional Strategies of Innovation).

References

1. Barriga BP, Mera KM, Fuentealba AJ, Seeman FP (1978) Effect of gamma irradiation (^{60}Co) on spring wheat seed (*Triticum aestivum* L.) at different storage times. Agro Sur 6:100–108
2. Calabrese EJ (2005) Paradigm lost, paradigm found: the re-emergence of hormesis as a fundamental dose response model in the toxicological sciences. Environ Pollut 138;3:379–411
3. Calabrese EJ, Baldwin LA (1993) Performing ecological risk assessment. Lewis Publisher, Chelsea, USA
4. Calabrese EJ, Baldwin LA (2002) Radiation hormesis and cancer. Hum Ecol Risk Assess 8;2:327–353
5. Calabrese EJ, Baldwin LA (2003) The hormetic dose-response model is more common than the threshold model in toxicology. Toxicol Sci 71;2:246–250

6. Fornalski KW, Dobrzyński L (2009) Ionizing radiation and health of nuclear industry workers. Int J Low Radiat 6;1:57–78
7. Fornalski KW, Dobrzyński L (2011) Pooled Bayesian analysis of twenty-eight studies on radon induced lung cancers. Health Phys 101;3:265–273
8. Fowler DB, MacQueen KF (1972) Effect of low doses of gamma radiation on yield and other agronomic characters of spring wheat (*Triticum aestivum*). Rad Bot 12:349–353
9. Kumagai J, Katoh H, Kumada T, Tanaka A, Tano S, Miyazaki T (2000) Strong resistance of *Arabidopsis thaliana* and *Raphanus sativus* seeds for ionizing radiation as studied by ESR, ENDOR, ESE spectroscopy and germination measurement: Effect of long-lived and super-long-lived radicals. Radiat Phys Chem 57;1:75–83
10. Kurimoto T, Constable JVH, Huda A (2010) Effects of ionizing radiation exposure on *Arabidopsis thaliana*. Health Phys 99;1:49–57
11. Luckey TD (2006) Radiation hormesis: the good, the bad and the ugly. Dose-Response 4;3:169–190
12. Luckey TD (2007) Documented optimum and threshold for ionizing radiation. Int J Nucl Law 1;4:378–409
13. Luckey TD (2008) Abundant health from radioactive waste. Int J Low Radiat 5;1:71–82
14. Luckey TD (2008) The evidence for gamma ray photosynthesis. 21th Century Science & Technology, Winter 2008, pp 4–6
15. Macklis RM, Beresford B (1991) Radiation hormesis. J Nucl Med 32;2:350–359
16. Majeed A, Khan AUR, Ahmad H, Muhammad Z (2010) Gamma irradiation effects on some growth parameters of *Lepidium sativum* L. J Agric Biol Sci (Asian Research Publishing Network) 5;1:39–42
17. Micco VD, Arena C, Pignalosa D, Durante M (2011) Effects of sparsely and densely ionizing radiation on plants. Radiat Environ Biophys 50:1–19
18. Miller MW, Miller WM (1987) Radiation hormesis in plants. Health Phys 52;5:607–616
19. Rennie DA, Nelson SH (1975) Low-dose irradiation of vegetable seeds: the effects on N and P uptake. Can J Plant Sci 55:761–769
20. Sheppard SC, Evenden WG (1986) Factors controlling the response of field crops to very low doses of gamma irradiation of the seed. Can J Plant Sci 66:431–441
21. Sheppard SC, Guthrie JE, Thibault DH (1992) Germination of seeds from an irradiated forest: implications for waste disposal. Ecotox Environ Safe 23;3:320–327
22. Sheppard SC, Regitnig PJ (1987) Factors controlling the hormesis response in irradiated seed. Health Phys 52;5:599–605
23. Simon J, Bhattacharrya S (1977) The present status and future prospects of radiation stimulation in crop plants. Budapest, Hungary: PHYLAXIA, Veterinary Biologicals and Feedstuffs
24. Sinkkonen A, Myyra M, Penttinen OP, Rantalainen AL (2011) Selective toxicity at low doses: experiments with three plant species and toxicants. Dose-Response 9;1:130–143
25. Sivia DS, Skilling J (2006) Data analysis. A Bayesian tutorial, 2nd ed. Oxford University Press, Oxford