The effects of X-rays in the range of diagnostic levels on the growth rate of pistachio seedlings

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Abstract

Background: The objective of this research was to assess the impact of low doses of diagnostic X-rays on the growth rate of pistachio seedlings.
Material and methods: Before cultivation, pistachio seeds underwent a soaking process in tap water for two days, which was succeeded by an additional five days of being wrapped in a damp cloth. Fifteen days into the cultivation process, the newly sprouted plants were subjected to X-ray irradiation. The plants received X-ray doses of 80 kVp and 80 mAs, with a distance of 100 cm maintained between the source and the seedlings, over a series of consecutive days. On the thirtieth day, the plants were extracted from the soil, and measurements were taken for stem length, stem diameter, root length, leaf count, and overall plant weight.
Results: The root lengths in the thrice irradiated group and the control group were recorded as 3.71 ± 3.14 cm and 2.96 ± 2.53 cm, respectively. The observed difference was not statistically significant ($P < 0.186$). Additionally, other parameters measured, such as stem length, stem diameter, number of leaves, and plant weight in the irradiated groups, exhibited variations when compared to the control group; however, these differences also did not reach statistical significance.
Conclusion: The findings suggest that diagnostic doses of X-rays, which involve very low energy levels, may influence the growth of pistachio plants. However, these purported biopositive effects are not particularly pronounced or significant. Nonetheless, the existing data appear insufficient, indicating that additional research is necessary to explore the potential hormetic mechanisms associated with these diagnostic energy levels.

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1. Introduction

Ionizing radiation has long been recognized as a contributor to detrimental biological effects. While significant adverse effects typically necessitate exposure to relatively high radiation doses, it is believed that mutations and cancer may arise from much lower levels of exposure. Many studies conducted over recent decades indicate that the impact of low doses of ionizing radiation cannot be accurately forecasted by extrapolating data derived from high doses. Instances of beneficial biological responses to low radiation doses, often called stimulating or hormetic effects, support this assertion [1]. The biological effects of low doses of ionizing radiation remain one of the most complex and contentious topics in radiobiology during the latter half of the 20th century. Historical research indicates that the harmful effects of high radiation levels were recognized shortly after the discovery of radiation itself, beginning with Roentgen's identification of X-rays in 1895 and Becquerel's subsequent discovery of radioactivity. Since the advent of X-rays and radioactive materials, scholarly opinions regarding the dangers posed by low doses of radiation have fluctuated between acceptance and skepticism. Moreover, a significant number of scientists worldwide argue that there is an irrational level of fear and anxiety surrounding this issue today. The pervasive presence of radiation, even at minimal levels, has led to a devaluation or neglect of radiation technology's substantial and beneficial applications [2]. The study of ionizing radiation has resulted in two contrasting theories about the effects of low doses. One theory argues that any exposure, no matter how small, carries a risk, claiming there is no safe threshold for biological effects. In contrast, the opposing theory asserts that harmful effects have a threshold and may even suggest benefits from low doses, referencing concepts like radiation hormesis and adaptive response [3,4]. Hormesis refers to the beneficial effects of low doses of substances that are toxic at higher concentrations. The hormesis hypothesis suggests that the dose of a substance is crucial in determining its positive or negative effects. Although the concept emerged from pharmacological research, various global studies indicate that low levels of ionizing radiation may also demonstrate hormetic effects [5]. In the 1950s, Luckey [6], a key figure in radiation hormesis, first observed that low doses of oral antibiotics could extend cattle lifespan. He later found that low-dose radiation might also induce hormetic effects. In 1980, he published the first comprehensive report on radiation hormesis [6]. The adaptive effect, known as Radiation Hormesis, posits that organisms exposed to low doses of radiation before higher doses experience less radiation damage than those exposed only to high doses. This mechanism is termed the radiation adaptive effect. Recent studies suggest this effect may also reduce malformations related to ionizing radiation exposure [7]. studies have shown that low doses of ionizing radiation can have a dual impact on cellular DNA: they may cause slight, dose-dependent DNA damage, but they may also trigger a protective adaptation that helps mitigate this damage [8]. Recent reports have emerged regarding the impact of low doses of radiation on the lifespan extension of insects [9]. The significance of research concerning the biological effects of low doses of ionizing radiation is further underscored by the potential acceptance of hormesis. Should this concept gain widespread recognition, the substantial expenses associated with protective measures against ionizing radiation in developed nations would be markedly diminished [10]. At the time of Luckey's preliminary investigations [6], numerous studies had been conducted on the hormetic effects of low-dose radiation across various living organisms, including plants. However, the reliability of these earlier studies is questionable due to issues such as the lack of reproducibility in findings and inadequate dose calculations [11]. There have also been observations of biopositive effects in plants following exposure to extremely high doses of irradiation. To the best of the authors' knowledge, there has been no published research regarding the positive biological effects of X-rays at doses typical of diagnostic radiology on pistachio plants. In 2003, Afifi et al. [12] examined the impact of ionizing radiation on the growth of apple mycoflora and the production of aflatoxin. Notably, their findings revealed that irradiation at a dose of 0.5 kGy enhanced aflatoxin production in all fungi tested (Aspergillus flavus, A. fumigatus, and Penicillium expansum), whereas increasing the radiation dose to between 1.5 and 2.5 kGy resulted in a decrease in aflatoxin production [12]. In 2004, Fan et al. [13] exposed alfalfa seeds to gamma radiation at varying doses ranging from 0 to 4 kGy. The seeds were then germinated and cultivated at a temperature of 23°C for 8 days. Their observations indicated that the sprouts derived from the irradiated seeds exhibited higher levels of antioxidants and ascorbic acid in comparison to those from non-irradiated seeds [13]. In that same year, Al-Salhi et al. [14] investigated the impact of gamma radiation on the biophysical and morphological traits of corn plants. The findings from their research indicated that corn exposed to radiation levels of 1.5 Krad and 2.5 Krad exhibited a notable enhancement in all measured growth parameters [14]. Zaka et al. [15] conducted a study in France examining the impact of short-term gamma radiation on pea plants. In this research, five-day-old seedlings were subjected to radiation doses ranging from 0 to 60 Gy. The researchers assessed the growth and development of the plants over two generations following the exposure. Their results indicated that doses exceeding 6 Gy markedly inhibited the growth and reproductive capabilities of the first generation of plants [15]. Mortazavi et al. [16] evaluated the impact of X-rays utilized in diagnostic radiology on the growth of pinto beans. Their findings indicated that the energy emitted by X-rays in this context has a beneficial biological effect on the growth of the plant [16]. Rezk [17] conducted a study on two distinct genotypes of the okra plant through the application of irradiation. The findings indicated that doses below 5 Gy resulted in beneficial biological effects on the examined factors, whereas doses exceeding 5 Gy led to adverse effects [17]. Jonathan [18] demonstrated that the irradiation of cereal crops at doses of 0.7 Gy and 1 Gy does not significantly affect root development in cereal plants [18].

2. Material and methods

Pistachio nuts represent one of the primary agricultural products cultivated in Iran, which accounts for over 50% of global pistachio production [19] is one of the most important nuts products in Iran especially in Rafsanjan County. So, we bought fresh pistachio seeds from the brand (Ahmed Aghaei) related to the collection of the year. We randomly separated 300 of the purchased seeds and divided them into three groups, each group including 100 seeds. Then, we subjected the first group of 100 as a control group without any exposure to an X-ray beam. The second and third groups, together with the control groups were soaked in water for two days

(24 hours) before cultivation and then disinfected using fungicides (mancozeb 80%) after that for 4 to 5 days, we placed them under a damp cloth and at a temperature of 20 to 30 degrees Celsius and waited until the pistachio seeds germinated and disinfected again with 80% Mancozeb fungicide [2]. We established the culture within a laboratory setting. After fifteen days from the initiation of cultivation at a depth of 1 cm, the newly developed plants from the second group (the twice-irradiated group) were subjected to X-ray irradiation. This was accomplished using a portable Siemens X-ray machine, which is manufactured in Germany, applying the maximum energy levels typically utilized in standard diagnostic radiology (specifically, under lateral radiographic conditions for the lumbar vertebrae, which includes 200 mA, 80 kVp, and a duration of 0.4 seconds). The distance maintained between the radiation source and the samples was 100 cm, and the irradiation occurred over two consecutive days. The third group underwent X-ray irradiation for three consecutive days under identical conditions as the second group. Following a growth period of one month, we assessed various growth parameters, including stem length, leaf count, stem diameter, and overall plant weight. Additionally, we extracted the plants from the soil to measure the length of the central root. To analyze the frequency distribution of the measured variables (plant components), we employed the non-parametric Kolmogorov-Smirnov test, while Levene's test was utilized to assess the homogeneity of variance among the variables across the control, twice-irradiated, and thrice-irradiated groups. Subsequently, one-way ANOVA was conducted to compare the average growth of the Ahmad Aghaei brand pistachio plants under varying X-ray irradiation conditions.

3. Results

The Kolmogorov-Smirnov non-parametric test indicated that the frequency distribution of the examined variables (plant components) conformed to a normal distribution ($P>0.05$). Additionally, Levene's test confirmed that the homogeneity of variance among the studied variables (plant components) was consistent across the control groups, as well as those subjected to two and three rounds of irradiation ($P>0.05$). The factors assessed in all groups, including the control group, the group subjected to two irradiations, and the group subjected to three irradiations, are presented in Table No. 1 and illustrated in Charts No. 1 to No. 5. The average number of leaves in the control group and the twice-irradiated group were recorded as 9.83±3.63 and 9.90±3.28, respectively, with this increase not reaching statistical significance. Furthermore, the measurements for stem length, root length, stem diameter, and plant weight in the twice-irradiated group were lower than those in the control group, although this decrease also lacked statistical significance.

The control group exhibited an average of 9.83±3.63 leaves, while the group subjected to triple irradiation had an average of 10.10±3.34 leaves. In terms of root length, the control group measured 2.96±2.53 cm, compared to 3.71±3.14 cm in the thrice irradiated group. Additionally, the plant weight for the control group was recorded at 0.46±0.39 g, whereas the irradiated group showed a weight of 0.50±0.38 g. These observations indicate an increase in the growth parameters of pistachio seedlings; however, the differences were not statistically significant. Regarding stem length, the control group had an average of 17.13±8.02 cm, while the irradiated group measured 16.23±7.95 cm. The stem diameter was recorded at 1.90±0.50 mm for the control group and 1.87±0.53 mm for the irradiated group, both of which were lower than the control group, with this decrease also lacking statistical significance.

While the thrice-irradiated group exhibited greater root length, leaf count, and plant weight in comparison to the control group, these enhancements were not statistically significant. Additionally, the stem diameter and stem length in the thrice-irradiated group were lower than those observed in the control group, with these differences also lacking statistical significance.

Table 1. Factors assessed in the control group, as well as in the groups subjected to two and three rounds of irradiation.

Factor	Control group (mean + SD)	Twice irradiated group (mean + SD)	thrice irradiated group (mean + SD)	P-Value
Stem length (cm)	17.13± 8.02	16.17± 6.90	16.23± 7.95	0.764 (NS)
Leaf numbers	9.83 ±3.62	9.90± 3.28	10.10± 3.34	0.899 (NS)
Stem diameter(mm)	1.90± 0.50	1.87± 0.52	1.87± 0.53	0.984 (NS)
Root length (cm)	2.96± 2.53	2.82± 2.68	3.71± 3.14	0.186 (NS)
plant weight (gr)	0.46± 0.39	0.42± 0.38	0.50± 0.38	0.595 (NS)

**NS: not significant

Charts 1 to 5 detail the measured factors categorized into groups.

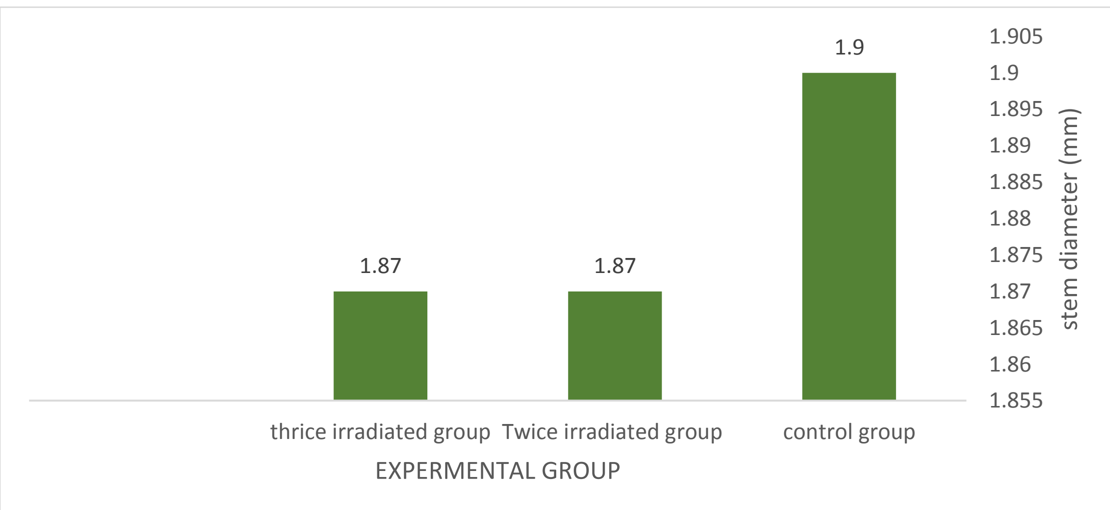

Chart 1. Stem diameter (mm) measured in groups

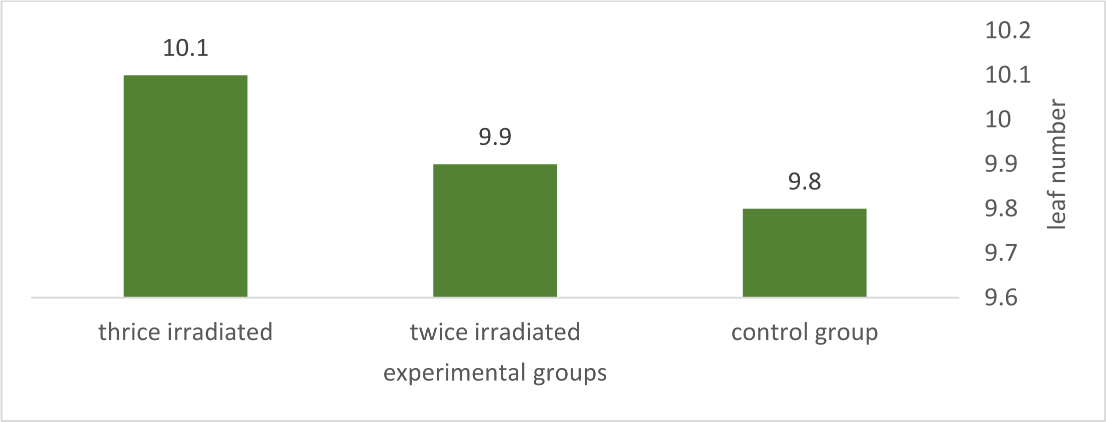

Chart 2. Leaf number in groups

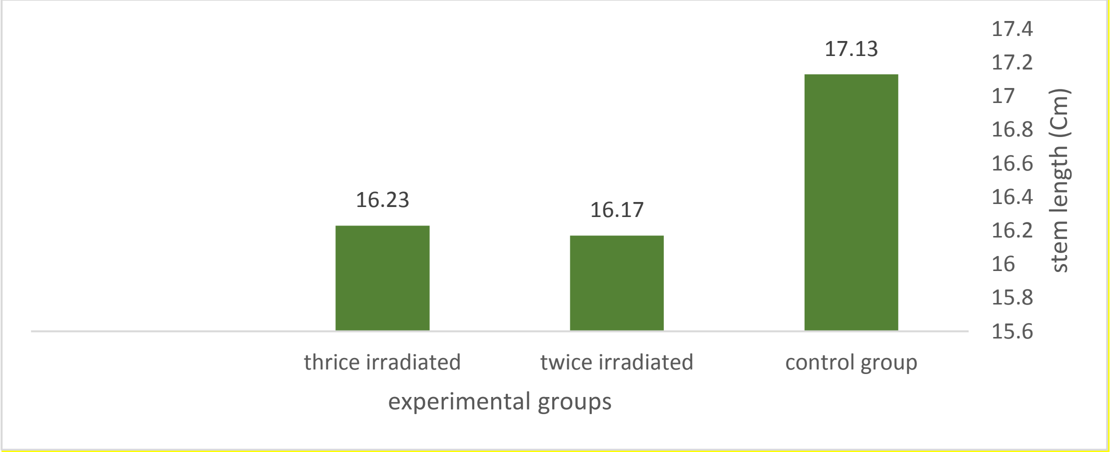

Chart 3. Stem length (cm) measured in groups.

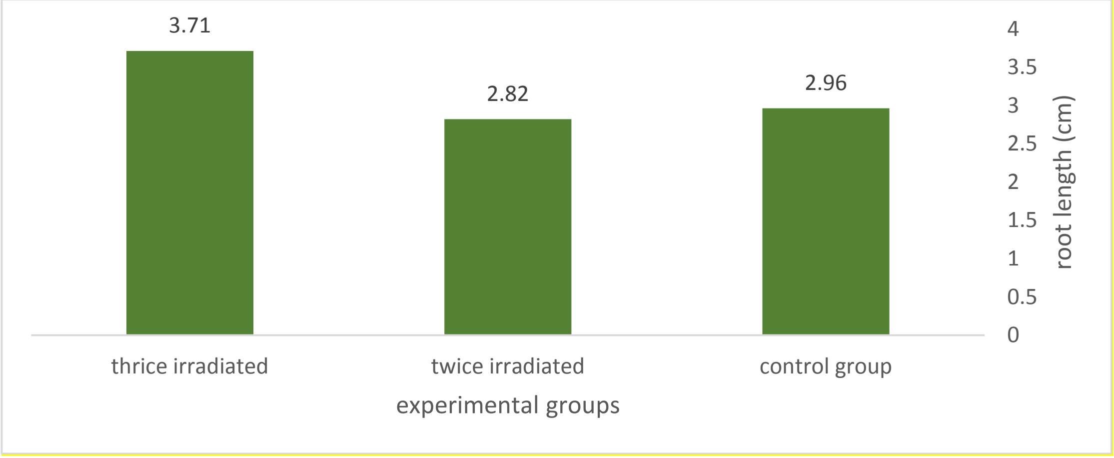

Chart 4. Root length (cm) measured in groups.

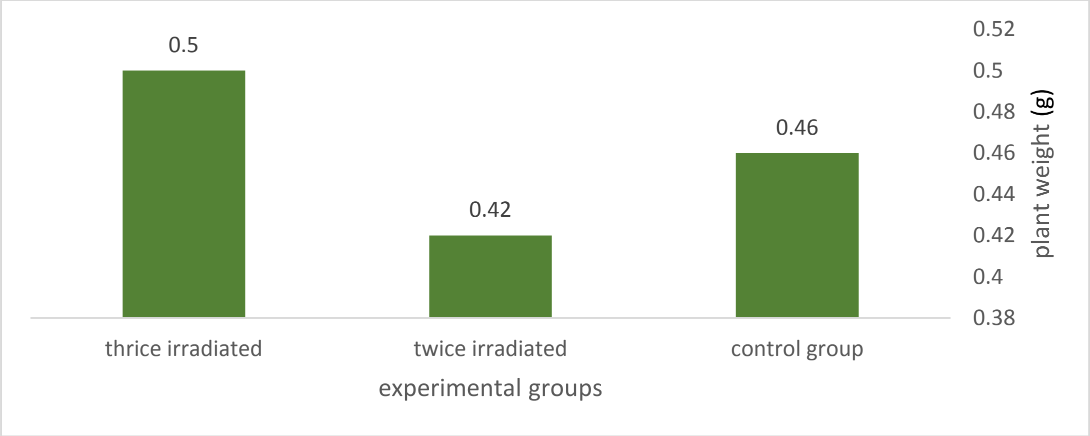

Chart 5. Plant weight (g) measured in groups

4. Discussion

Research findings exhibit significant variability, ranging from growth stimulation at lower doses to adverse effects at elevated doses [20]. The objective of this research was not to challenge the findings of other scholars in the domain of radiation hormesis; however, it is evident that variations in research methodologies employed by different investigators are resulting in disparate outcomes. The findings of this study indicated that while X-rays at the energy levels employed in diagnostic radiology exert some influence on the growth factors of the pistachio plant, these effects do not reach statistical significance. It is possible that further investigation in this area, along with increased repetitions of radiation exposure in the examined groups, may reveal positive biological effects on this plant. The slight enhancement of certain growth factors in the group subjected to three rounds of irradiation suggests a degree of stimulation consistent with hormetic effects. The induction of hormetic effects can be affected by various factors, including the type of plant, the research design, and, notably, the depth of seed cultivation, particularly at very low X-ray energy levels, a consideration that is often overlooked in numerous studies. The lack of stimulation of hormetic effects in plants subjected to double irradiation, along with only a slight increase in root length observed in the group exposed to three rounds of radiation, underscores the existence of a threshold dose necessary for the induction of hormetic effects following irradiation. While previous studies have explored the occurrence of positive biological effects in plants, to the best of the authors' knowledge, this research represents one of the initial investigations into the positive biological effects of X-rays within the realm of diagnostic radiology on the pistachio plant. Zaka et al. [15] similarly failed to demonstrate any significant hormetic effect in chickpeas. Conversely, researchers employing non-diagnostic doses have reported the presence of hormetic effects in certain plant species. For instance, Fan et al. [13] found that irradiated alfalfa seeds exhibited higher levels of ascorbic acid and antioxidants compared to their non-irradiated counterparts. Additionally, Al-Salhi's findings [14] indicated that significant alterations in all growth parameters were observed in corn seeds irradiated at doses ranging from 1.5 to 2.5 Krad. These studies, including those by Al-Salhi [14] and Fan [13], suggest that irradiation at levels exceeding diagnostic energy thresholds can affirm the hormetic effect of radiation on plant growth factors. However, Mortazavi's 2006 results, which indicated a hormetic effect of X-ray radiation on the growth factors of Pinto beans, present a compelling case for further research. This suggests that researchers should pursue investigations involving various irradiation procedures at higher doses or energy levels, alongside different cultivation methods and plant species, to uncover more pronounced bio-positive effects of radiation on plants.

5. Conclusion

Soil compaction and moisture levels significantly decrease the dry weight of roots and shoots, the shoot-to-root dry weight ratio, root length, and rooting depth [21]. The lack of measurements for these factors, along with their effects on the experiment, may limit our study. Overall, our findings suggest that X-rays used in diagnostic radiology can promote the growth of certain plant species. The slight but notable increase in root length observed in plants exposed to triple irradiation in this study is significant and merits further exploration. These results align with several previous studies using diagnostic levels, but differ markedly from findings related to non-diagnostic doses, thus confirming the presence of hormetic effects in plants exposed to higher doses. The depth at which seeds are planted in the soil, specifically 1 centimeter as noted in this study, is a crucial yet often overlooked factor in many research efforts. This depth significantly affects the radiation penetration in the soil, influencing study outcomes. The current information is incomplete, suggesting a need for further comprehensive studies to validate these findings and explore additional mechanisms by which radiation may affect plant growth.

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Conflict of Interest

None of the authors of the present study declared a conflict of interest.

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