

Effect of different Gamma Rays doses on Seed Germination, Seedling parameter on local seed okra (*Abelmoschus esculentus* (L.))

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Received:
24/10/2023

Revised:
05/11/2023

Accepted:
24/03/2024

Published:
30/03/2024

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Citation: Hassoon, W. H., Dheyab, N. S., & Abd Alsada, A. J. (2024). Effect of different Gamma Rays doses on Seed Germination, Seedling parameter on local seed okra (*Abelmoschus esculentus* (L.)). *Journal of agricultural, environmental and veterinary sciences*, 8(1), 84 – 92.
<https://doi.org/10.26389/AJSRP.W241023>

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Abstract: The experiment was carried out to study the effect of different gamma irradiation in 100 -800 Gy with non-irradiated control on seed okra [*Abelmoschus esculentus* (L.)]. The study carried in Microbiology Dept. Directorate of Agricultural Research. Ministry of Science and Technology in 2020 .The results showed that 100-300 Gy had positive effects with respect to all studied growth seeding parameters. With increase in radiation above 300 Gy a decreasing germination potential, The lowest germination percentage was recorded at 800Gy, speed of germination was noted by Control (10.00 day) followed by gamma irradiation dose 800(9.90 day) and the same dose was logged lowest mean daily germination (7.85 seed day-1), higher doses (800,700,600) Gy marked reduction in root, shoot length, vigor index, chlorophyll a chlorophyll b .and total chlorophyll. The result showed a decreasing of the fresh weight of okra when radiation dose was increased While, dry weight was increasing when the radiation dose was increased. This study shows that the low dose gamma radiation may enhance the germination rate and the seedling growth during the early seedling state of okra seeds.

Keywords: Gamma Irradiation, Germination Percent, Vigor Index, Chlorophyll. Interdiction, *Abelmoschus esculentus*

تأثير جرعات مختلفة من اشعة غاما في انبات بذور ومؤشرات الانبات (*Abelmoschus esculentus* (L.) المحلية الباميا المحلية في بذور الباميا المحلية)

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المستخلص: تم تنفيذ تجربة لدراسة تأثير اشعة كاما في انبات ونمو بذور الباميا المحلية. البذور عرضت لجرعات مختلفة من اشعة كاما مستخدم مصدر كاما كوبلت 60. تم تشيع بذور الباميا بجرعات من 100-800 كري بفواصل 100 كري بين جرعة واخرى مقارنة مع معاملة السيطرة بدون تشيع تحت ظروف مسيطر عليها. تم تسجيل الملاحظات حول مؤشرات الانبات والنمو مثل نسبة وسرعة الانبات ومعدل اليومي للانبات وطول الاوراق والجذور والنسبة المئوية لطول الجذر على الاوراق والوزن الرطب والجاف للدبابات وقوة البذور وكوروفيل أ وب والكلبي. في مرحلة الانبات اكدت النتائج ان الجرعات من 100-300 كري كانت لها اثار ايجابية في جميع مؤشرات الدراسة، لكن بارتفاع جرع التشيع فوق 300 كري تنخفض مؤشرات الانبات. مما يثير الاهتمام معاملة التشيع 800 كري سجلت اقل نسبة انبات (79.67%) واعلى سرعة انبات لوحظت في معاملة السيطرة (10 يوم) والتي لم تختلف معنويا عن المعاملة 800 كري (9.90 يوم) والاخرة سجلت اقل معدل يومي للانبات (7.85 بذرة يوم⁻¹). الجرعات العالية (800، 700، 600) كري ادت الى نقصان في طول الجذر والاواق وقوة البذور وكوروفيل أ وب والكلبي. اكدت النتائج انخفاض في الوزن الرطب بارتفاع جرع التشيع بينما زيادة الوزن الجاف بارتفاع الجرع التشيع. اظهرت نتائج الدراسة ان الجرع المنخفضة من اشعة كاما حفزت مؤشرات الانبات والنمو في المراحل الاولى للانبات في بذور الباميا.

الكلمات المفتاحية: إشعاعات غاما، نسبة الإنبات، المؤشر الحيوي، الكلوروفيل، بذور الباميا

Introduction

Okra (*Abelmoschus esculentus*) is a member of the Malvaceae family and ranks as the sixth most popular vegetable crop, cultivated extensively across various climatic conditions throughout Iraq. Its nutritional profile includes carbohydrates, proteins, fats, vitamins, and significant amounts of calcium and phosphorus (Swamy, 2023). India stands as the largest producer of okra, with cultivation spanning 3.8 lakh hectares and an annual yield of 36.84 lakh tons (Yogeshwari et al., 2021). Despite its diverse uses, current okra production levels struggle to meet the rising demand attributed to population growth (Haq et al., 2023). Enhancing crop traits through induced mutations using gamma rays is a well-established practice. Gamma rays are renowned for their exceptional penetrating power, surpassing that of alpha and beta rays, making them a highly effective form of radiation (Riviello et al., 2022). Furthermore, numerous studies, including research by Singh and Datta (2010), have shown that gamma rays are more economical and successful in achieving desired outcomes. There are several usages of gamma rays in agriculture to improve the characters and productivity. Seed irradiation might cause physiological changes such as stimulate of seed germination, shoot and root elongation (Villegas et al., 2023). Or biological effect includes changes molecules in the cell by produce free radicals (Marcu et al., 2013). These effects depending on the gamma radiation dose and duration (Lowe et al., 2022). Seed irradiation with high doses of gamma rays disturbs the synthesis of protein and DNA, hormone balance, leaf gas-exchange, water exchange and enzyme activity Villegas et al., 2023). While the low-dose irradiation did not cause these changes in the ultra-structure of chloroplasts. Some studies confirmed the effects of greater exposure of gamma rays changes in the plant cellular structure and metabolism, dilation of thylakoid membranes, alteration in photosynthesis, modulation of the anti-oxidative system (Mounir et al., 2022) while low doses of gamma rays stimulate all studied growth parameters of okra (Hegazi and Hamideldin, 2010). Moreover, The aim of this study the effect of gamma irradiation by different doses treatments on germination, seedling parameter and chlorophyll on local seed okra. Such a study is needful to unveil any desirable features for agronomic and find a better dose gamma radiation to enhance the germination rate and the seedling growth during the early seedling state of okra seeds.

Materials and Methods

The experiment was carried out as per Completely Randomized Design (CRD). Seeds of cvs. Local Hassinwee of okra (*Abelmoschus esculentus*) were selected for irradiation. Moisture content of the seeds was adjusted at 13% before irradiation. Gamma irradiation was conducted using 60 Co gamma source at a dose rate of 0.864 kGy/h in International Atomic Energy Agency (IAEA). Okra seeds were irradiated with 100-800 Gy by 100 Gy intervals and non-irradiated seeds as control. The irradiated seed along with non-irradiated control were sown in petri dishes in the laboratory. 25 seeds from each treatment were sown in Petri dishes containing each 5 ml of distilled water. Petri dishes were placed in an incubator for 14 days at 25°C. Number of germinated seeds was recorded for 6 days. The germination percent was calculated as follows:

$$GP\% = \frac{\text{Number of germinated seeds after 6 days}}{\text{Total number of seeds}} \times 100 \quad (\text{hassoon, 2014})$$

To assess the speed of germination,

The mean germination time (MGT) was calculated as follows (Khaje-Hosseini *et al.*, 2003):

$$MGT = \frac{\sum (fx)}{\sum f}$$

where MGT is mean germination time, f is the number of newly germinated seeds on each day and x in the day of counting. U.S.A).

Two weeks after sowing, root and shoot length (cm), root/shoot ratio and seedling fresh and dry weight were recorded. The seedling vigor index was calculated according to the following formula (Tao et al., 2023):

$$\text{Seedling Vigor index} = \text{seedling length (cm)} \times \text{germination percentage}$$

Determination of photosynthetic pigments chlorophyll *a* and *b* at 646 and 663 nm leaves were estimated in 80% acetone extracts according to the following equation and expressed in milligram per gram fresh weight of plant material (Kiong, 2008):

$$\text{Chlorophyll } a, \text{ Chl } a = 12.25 (\text{OD}_{663}) - 2.79 (\text{OD}_{646})$$

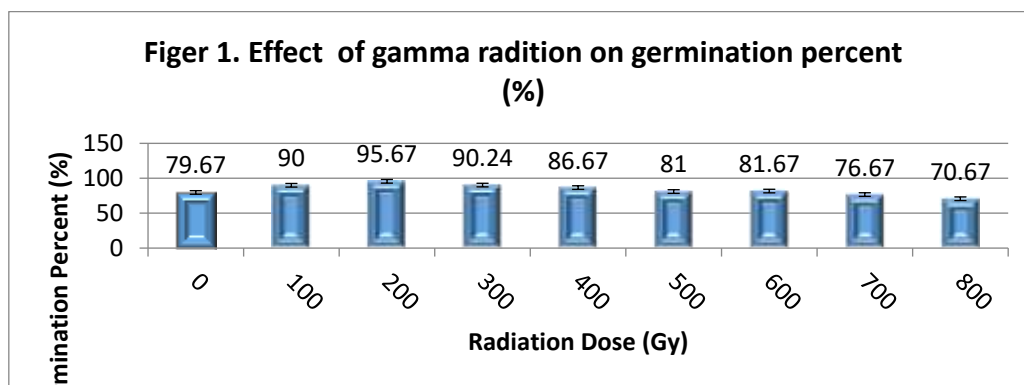
$$\text{Chlorophyll } b, \text{ Chl } b = 21.50 (\text{OD}_{646}) - 5.10 (\text{OD}_{663})$$

$$\text{Total chlorophyll, Chl } a + \text{ Chl } b = 7.15 (\text{OD}_{663}) + 18.71 (\text{OD}_{646})$$

Statistical analysis: The experimental design was a completely randomized factorial. The factors were gamma irradiation (8 levels) with three replications. ($P < 0.05$) was used to determine the differences in average of all tested parameters between irradiated and non-irradiated plantlets. Statistical analysis was performed using Gestate version 12.

Results

The data presented in figure -1 that germination percent was significantly increase by lowest doses of gamma irradiation and maximum germination (95.67%) was showed at 200 Gy followed by gamma irradiation dose 100 Gy (90.00%).The lowest germination percentage was recorded at 800Gy followed by 700 and control recording 70.67,76.67 and 79.67 percent respectively.



Differences among treatments in respect of speed of germination were highly significant. However, the maximum speed of germination was recorded by gamma irradiation dose 300Gy (5.5 day). The lowest speed of germination was recorded by Control (10.00 day) followed by gamma irradiation dose 800 (9.90 day) and 700 Gy (9.33day). (Table-1) (Figure2.)

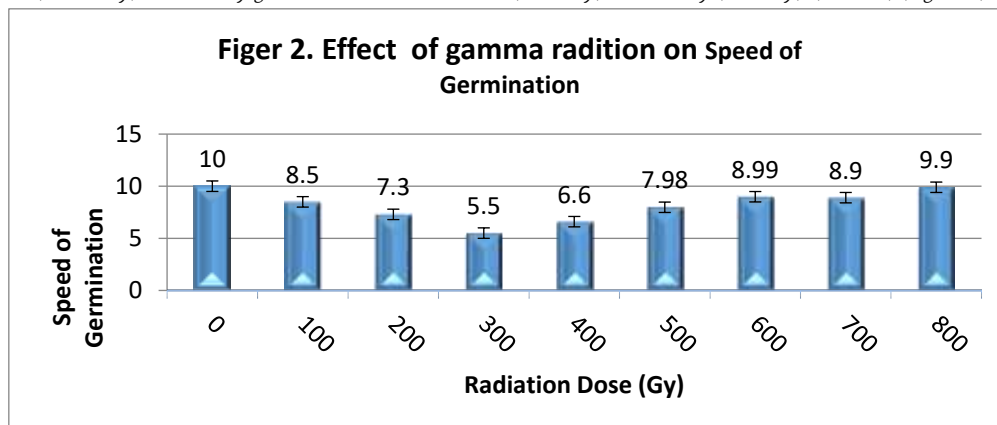
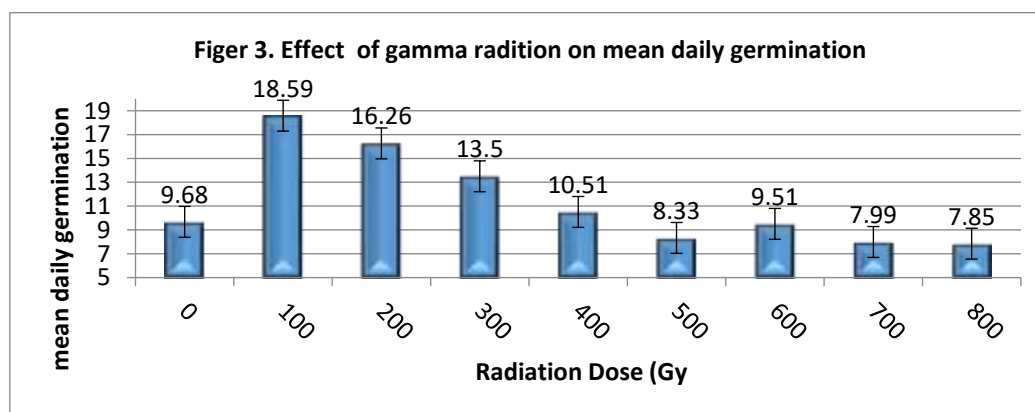
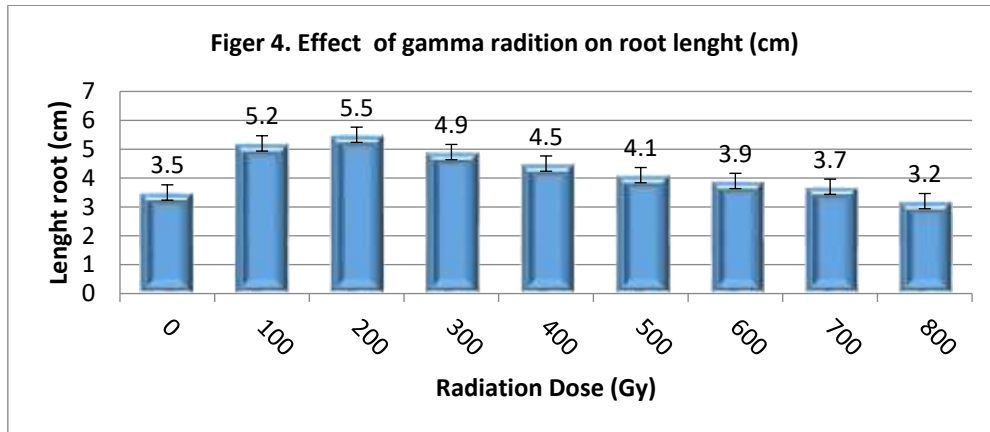


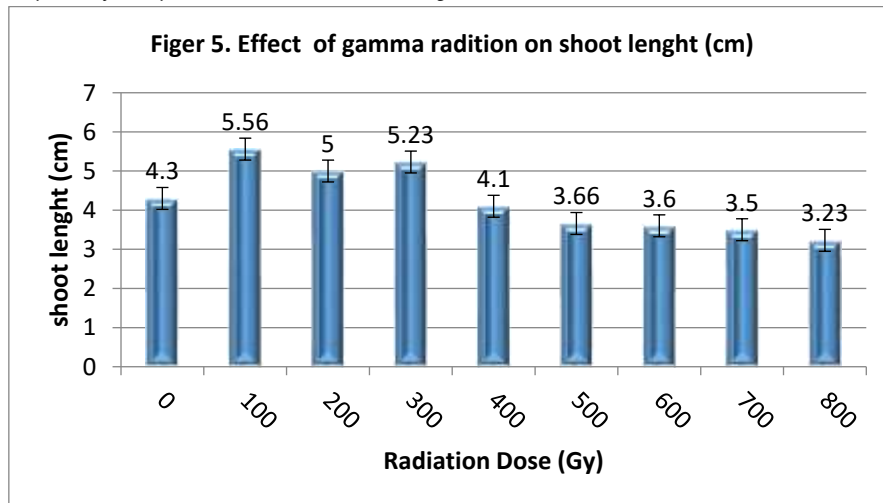
Table-1 were observed the significant differences in mean daily germination in all gamma irradiation doses. The best treatmeant was recorded highest mean daily germination by 200 Gy (16.26 day) whereas, radiation dose 800 Gy (7.85) was logged lowest mean daily germination (Figure.3).



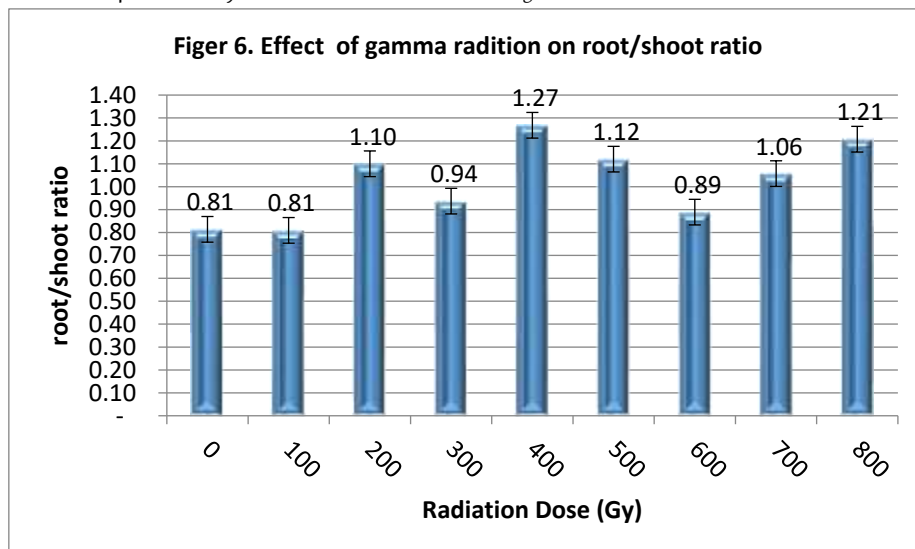
Effect of gamma radiation on root and shoot length in this study was in low dose stimulate growth seedling parameters while higher doses marked reduction in root and shoot (Table-2) . The highest length of root (5.5cm) was observed at 200 Gy and 100 Gy had no significant difference in root length and less root length was showed 800 Gy (3.2cm) figure 4 .



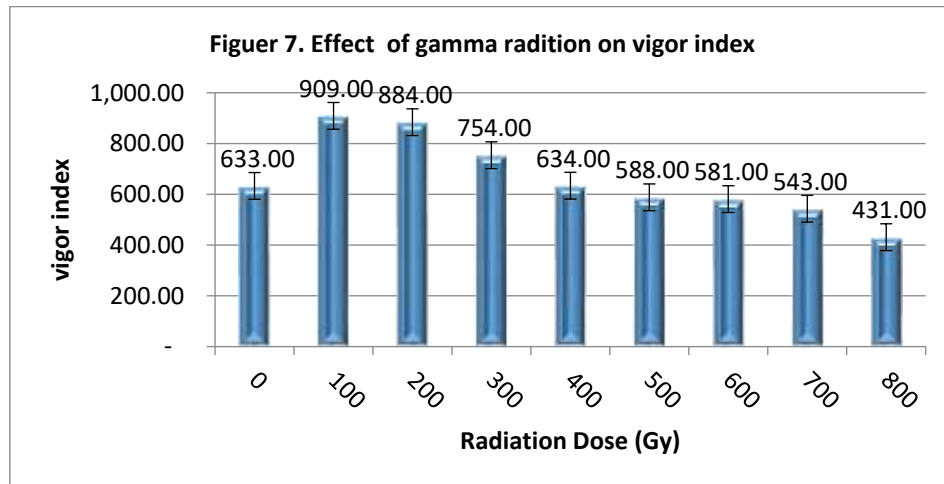
The maximum shoot length was observed for treatment of 100,300 and 200 Gy (5.56, 5.23 and 5.0 cm) when seed okra was exposed by low do gamma ray. By increasing radiation dose to 400, 500,600, 700 and 800 Gy shoot length declined 4.10,3.66, 3.60 3.5 and 3.23 respectively compared to the control (4.3 cm) figure 5 .



The results of the present study showed that the root/shoot length ratio was not relative to the increase in dosage (Figure.6).



The results of the figure 7 showed that the vigor index was decreased in decreasing order by the increase of irradiation dose. However, 100 Gy recorded highest vigour index 909.00 (Table-2) The gradual increase in gamma dose in corresponding decrease in vigour index from 909.00 at 100 Gy to 431 at 800 Gy.



Data in Table 3 figure 8 shows irradiation treatment 100 Gy exhibited highest chlorophyll-a, chlorophyll-b and total chlorophyll concentration in leaves of 0.57, 0.50 and 1.06 mgg^{-1} respectively. The treatment least chlorophyll-a, chlorophyll-b and total chlorophyll concentration were content recorded with 800Gy (0.19,0.07 and 0.25 mgg^{-1} respectively). The results obtained are in conformity with the findings of kim *et al.*2004 in red pepper.

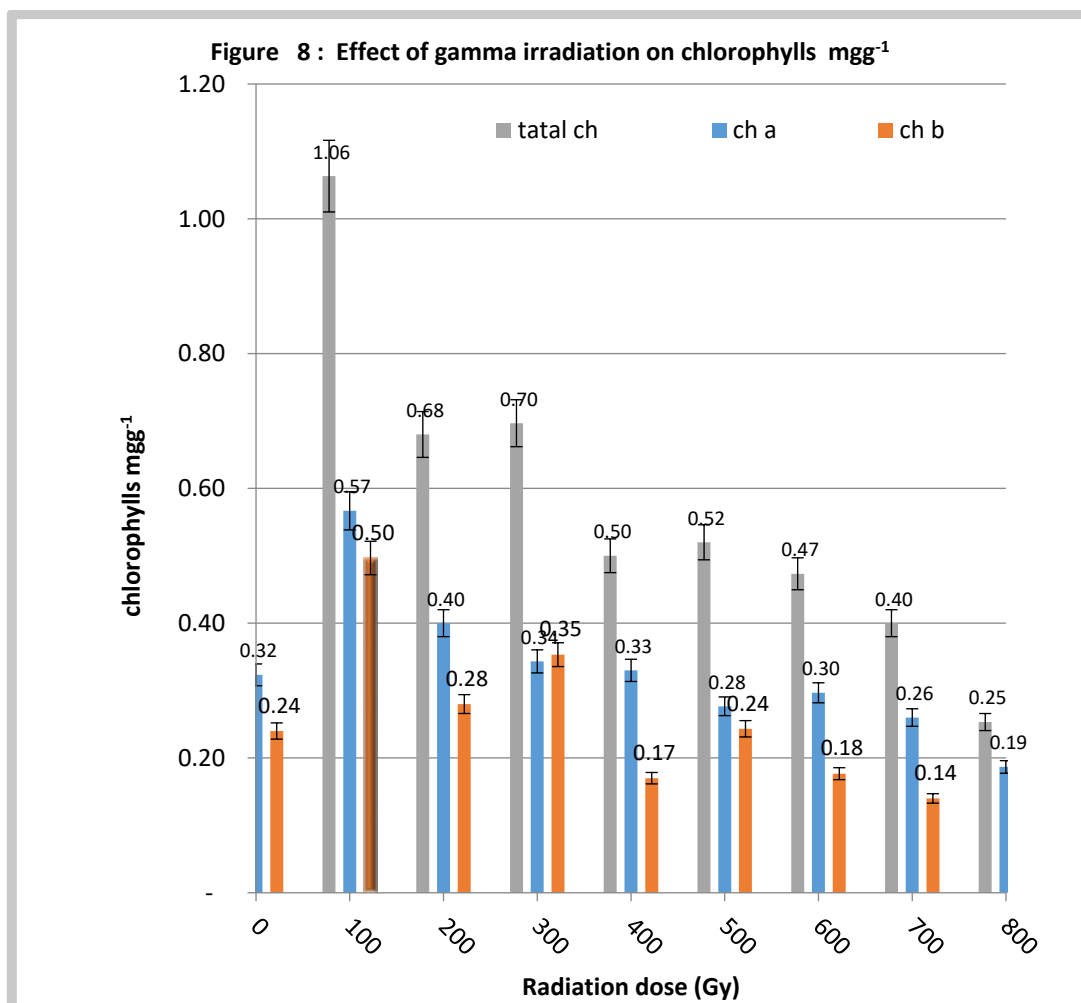
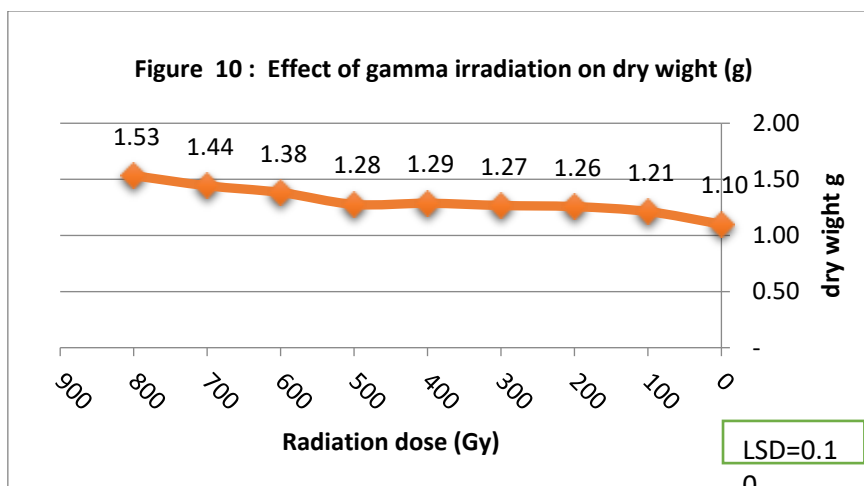
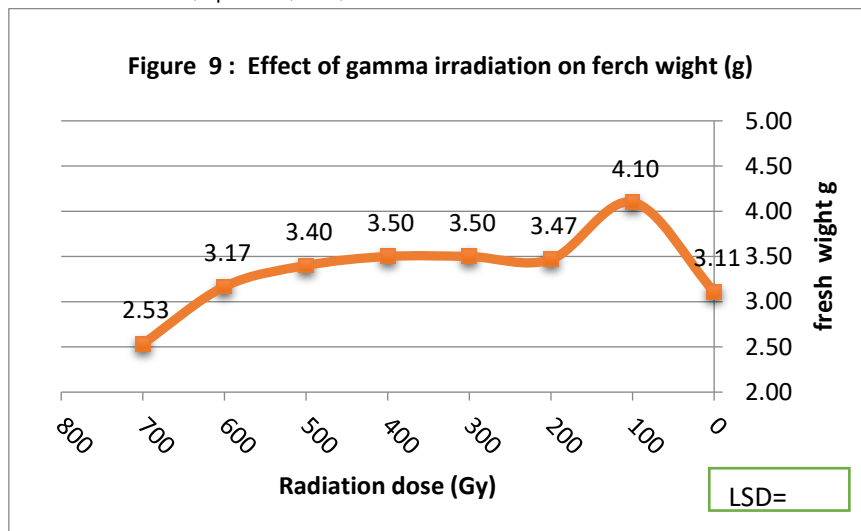


Table.3 Effect of gamma irradiation on chlorophylls of okra

Radiation Dose (Gy)	Chlorophylls (mg g ⁻¹)		
	Chlorophyll-a	Chlorophyll-b	chlorophyll Total
0.00	0.32	0.24	0.56
100	0.57	0.50	1.06
200	0.40	0.28	0.68
300	0.34	0.35	0.70
400	0.33	0.17	0.50
500	0.28	0.24	0.52
600	0.30	0.18	0.47
700	0.26	0.14	0.40
800	0.19	0.07	0.25
Grand mane	0.33	0.24	0.57
LSD 0.05	0.15	0.25	0.37

The fresh and dry weight of okra were shown in figure 9 and 10. The result showed a decreasing of the fresh weight of okra when radiation dose was increased. Okra's dry weight was increasing when the radiation dose was increased figure 10. The results showed that increasing dosage from 100 to 800 Gy reduced on fresh weight Figure 9 the decrease in fresh weight with increase in gamma radiation is in agreement with results of Jaipo et al (2019) on okra.



Discussion

Several germinating parameters are used in early assessment of effectiveness of irradiation to stimulate seed germination and induce mutations. Seed germination percent, speed of germination and mean daily germination have been used extensively. The superiority of 100,200 and 300 Gy irradiation dose in stimulating germinating parameters. The enhancement effects of gamma radiation on germination may be attributed to activity enzyme germination like amylase and protease or to protein synthesis activation, which occurs during the early stage of germination (Amir et al ,2017). Low dose of gamma rays might have induced the growth stimulation signals that triggered the biosynthesis of antioxidants and hormones (Hebat-Allah, 2022) These results are in agreement with Wi *et al.* (2007) whose noticed that treating seeds with low dose of gamma will induce a hypothesis and growth stimulation by increasing the antioxidative capacity of the cells to easily overcome daily or changing the hormonal signaling network in plant cells . Higher exposures of gamma rays may cause injury in seeds (Hong *et al.*, 2022) or maybe occur due to cytogenetic damage, physiological disturbance, and disturbance in balance between inhibitors of growth regulators and promoters (Yasmin and Chandran,2022). Speed of germination offers best index of seed vigor which would facilitate in theorizing the strong and weak seedlings. The Speed of germination decreased significantly at 300 Gy (5.5 days) as compared to control (10 days). Thus, it was observed that gamma irradiation increased days to speed of germination. the mean daily of germination was positively correlated with Oxygen uptake, Dehydrogenase activity by providing energy to the germinating embryo and interfering with integrity and overall capacity of the metabolic machinery of the young germinating primordial. (Aparna *et al.*,2023)

The physiological changes induced by gamma irradiation and effected growth and development of plants (Al-Bachir, 2014). Increased seedling parameter like root and shoot length might be attributed to ionizing radiation. low dose irradiation was essential to activate metabolic changes at cellular level. While higher dosage of irradiation reduced mitotic activity of meristematic tissues and caused root development to be postponed giving poor root (Suneetha *et al.*,2018).

Pribil et al. (2014) believed the low dosage of gamma irradiation stimulated chlorophyll content by the integrity of thylakoid membrane which remains unaffected at lower dosage. The cellular membrane had a lot of important membrane proteins to which are several integral membranes sets and light absorbing pigments, most notably chlorophylls. Correspondingly, Higher dosage of gamma irradiation was induce change physiological and biochemical alteration in plants like thylakoid membrane , water exchange , hormonal balance, enzyme activity and leaf gas-exchange (Pushparajan,2014).These effects of gamma irradiation induce change in cellular structure and metabolism in plant such as dilation of thylakoid membranes, accumulation of phenolic compounds, variation of the antioxidant system and modification in photosynthesis .the chloroplasts was sensitive to dosage of gamma radiation, mostly of thylakoid membranes being heavily swollen (Shala,2019).The result showed a decreasing of the fresh weight of okra when radiation dose was increased at 100 GY treatment, the fresh weight much higher than other doses . Similarly, Also Benjawan et al. (2007) concluded that low doses of gamma irradiation increased effect on plant height, dry weights of okra. The inhibitory effects were due to physiological disturbances on okra by ionizing radiations with imbalance of growth hormones while Okra's dry weight was increasing when the radiation dose was increased (Figure 10) Seeds exposed to higher doses maybe produced dwarf plants with reduced root and shoot length table 2.

Conclusion

Gamma irradiation can be used for enhancing the germination and seedling parameter and increase some biochemical compound, such as chlorophyll. The current study revealed that gamma irradiation at a dose level from 100 -300 Gy enhanced the growth parameters. It could be concluded that low doses of gamma irradiation have stimulating effect on seed okra. While increasing irradiation dose level 400 – 800 Gy inhibit seedling growth.

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