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The Effect of Gamma Radiation (^{60}Co) on the Germination of Chili Pepper (*Capsicum frutescens* L.)

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Abstract
This study aimed to investigate the effect of various doses of gamma radiation (60Co) on the germination of Prentul chili pepper. This research was conducted at the experimental field or greenhouse of the University of 17 August 1945, Banyuwangi, from August to September 2025. This study employed an RCBD with a single factor, namely the dose of ^{60}Co gamma radiation. The treatment consisted of 11 radiation dose levels: 0 Gy (control), 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 Gy. The data obtained were analyzed using analysis of variance (ANOVA) to determine the effect of radiation dose on each parameter. If significant differences were found, further analysis was conducted using Duncan's Multiple Range Test (DMRT) at a 5% significance level. Co-60 gamma radiation significantly affected the viability, mortality, germination rate (MGT), and early growth of prentul cayenne pepper seedlings. Low to moderate doses (50–200 Gy) reduced viability, whereas moderate doses (250–400 Gy) had a stimulatory effect similar to the control. The highest mortality occurred at a dose of 200 Gy, which is close to the LD_{50}, thus potentially generating genetic diversity. At high doses (>400 Gy), MGT increased sharply, and seedling growth was severely inhibited by physiological damage. Thus, the dose range of 200–300 Gy can be considered the optimum dose for mutation induction in prentul cayenne pepper.

Keywords: *Capsicum Frutescens* L.; Gamma Radiation 60Co; Germination; Seed Viability

Introduction
Chili peppers (*Capsicum frutescens* L.) are an important horticultural commodity in Indonesia, boasting economic value and high market demand. This commodity serves not only as a staple for household consumption and the food industry, but also as a primary source of income for farmers. However, national chili peppers productivity remains unstable due to various constraints, such as pest and disease attacks, climate change, and the limited availability of superior varieties with high adaptability and resistance to environmental stress. This is evident in data presented Badan Pusat Statistik (2025), which states that chili peppers production in Indonesia in 2022 was 15,444,409 quintals, then in 2023 there was a decline in cayenne pepper production to 15,067,621 quintals. In 2024, production increased again to 15,687,569.77 quintals.

Considering the fluctuating annual production of chili peppers due to the aforementioned constraints, innovation in plant breeding programs is needed to produce more productive, adaptive, and high-quality chili pepper varieties. Conventional breeding is generally used to improve plant traits, but this method is often time-consuming and limited by the availability of genetic diversity in germplasm. Therefore, the mutation induction approach is an effective

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alternative for creating new genetic diversity more rapidly. One widely used mutagenic agent is Cobalt-60 (60Co) gamma radiation, which has high energy and strong penetration power, capable of causing changes in plant DNA structure. The resulting mutations can be beneficial if they produce genetic variations that increase plant growth, resistance, or productivity. Suwarno et al. (2013) stated that gamma rays are one of the electromagnetic rays produced by radioactivity or nuclear reactions. Gamma radiation provides high energy, which can cause genetic mutations in plants. Gamma rays administered to planting material will result in plants with different yields than their parents. Aprianti et al. (2024) also stated that these changes have the potential to produce new genetic variations that can be used to improve plant traits and also develop new characteristics.

The effects of gamma radiation on plants depend on the dose administered. Low doses tend to be stimulative, for example, increasing metabolic activity, accelerating germination, and enhancing early growth (Sholikhah et al., 2024), while high doses can damage cell tissue, inhibit growth, and even cause seed death (Mounir et al., 2022). Therefore, determining the optimum dose is crucial for achieving a balance between stimulative and damaging effects.

The germination stage is the most critical phase in the plant life cycle and is often used as an early indicator to assess seed viability and the physiological response of plants to mutagen treatments. Although gamma radiation (^{60}Co) has been widely used in medicinal breeding, previous studies on various Capsicum species have shown varying responses to different radiation doses, particularly in early growth parameters. This inconsistency indicates that each variety has varying levels of radiosensitivity and requires specific testing. To date, information on how gamma radiation affects the germination stage of the Prentul chilli pepper variety is still very limited. However, observing parameters such as germination percentage, growth rate, Mean Germination Time (MGT), seedling vigor, and mortality are crucial for identifying radiation doses that seeds can tolerate without significantly reducing their viability. This data gap highlights the need for systematic research to determine a dose range that is not only safe but also has the potential to generate beneficial genetic variation for breeding programs.

Based on the background above, the problem formulation in this research is (1) How does Co-60 gamma radiation affect the viability, mortality, Mean Germination Time (MGT), and initial growth of prentul chili pepper seedlings? (2) What is the optimum dose that has the potential to be used as a physical mutagen agent to support chili plant breeding programs

Literature Review

Plant breeding is an applied scientific discipline that aims to improve the quality and quantity of commodities through the manipulation of genetic variation, using both conventional and biotechnological methods. Modern approaches combine traditional techniques (selection, crossbreeding) with molecular and in-vitro techniques (marker-assisted selection, genome editing, tissue culture, induced mutation) to accelerate the development of superior varieties while maintaining genetic diversity. This integration increases breeding efficiency but requires greater facilities, costs, and expertise than traditional methods (Sun et al., 2024).

There are several ways in plant breeding to increase genetic diversity, one of which is mutation breeding through gamma rays. Various studies on Capcisum have shown morphological and agronomic responses to gamma irradiation (changes in plant height, fruit number, chlorophyll mutations, etc.). Research in Indonesia reported various effective dose ranges, for example: 50-500 Gy or 100 Gy as a starting point for some varieties, so it is important to conduct radiosensitivity tests for prentul genotypes before determining the final dose range. International literature also supports the use of 60Co for mutation induction in horticultural crops (Sari et al.,

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2020)

^{60}Co gamma ray radiation has highly dose-dependent effects on chili pepper plants. At low to moderate doses, hormesis effects often occur, such as increased germination, early vigor, accelerated flowering, and improved vegetative growth. However, at higher doses, negative symptoms such as decreased viability, stunted growth, leaf deformation, reduced flower and fruit numbers, and even cell death begin to appear if the tolerance threshold is exceeded. Shamsiah et al., (2022) reported that doses below 100 Gy are still safe and stimulating, while doses of 200–400 Gy begin to cause adverse physical effects. Tia et al., (2021) also found that the critical dose (LD50) for cayenne pepper reportedly ranges from 150–300 Gy, indicating the need to determine an optimal dose range specific to the prentul variety.

^{60}Co gamma ray radiation allows for the emergence of phenotypic and genetic variations useful for breeding. Exposure to medium doses (200–400 Gy) can produce mutants with superior agronomic traits such as increased fruit number, larger fruit size, or stress tolerance. However, not all of these mutants are beneficial; further selection in the M1 and M2 generations is necessary to ensure genetic stability (Ahyar, 2020).

In the study by Marcu et al. (2013) entitled Effects of Gamma Radiation on Seed Germination, Growth and Pigment Content, and Free Radical-Induced ESR study in maize (Zea mays), the aim was to determine the effects of gamma radiation on plant germination, growth and development, and biochemical characteristics of maize. Dry maize seeds were exposed to a gamma source at doses ranging from 0.1 to 1 kGy. Our results showed that germination potential, expressed through the percentage of final germination and germination index, as well as physiological parameters of maize seedlings (root and shoot length) decreased with increasing irradiation dose. In addition, plants derived from seeds exposed to higher doses (≤0.5 kGy) did not survive beyond 10 days. Biochemical differences based on the content of photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids) revealed an inverse relationship with the exposure dose. Furthermore, the concentration of chlorophyll a was higher than that of chlorophyll b in irradiated and unirradiated seedlings. Electron spin resonance spectroscopy used to evaluate the amount of free radicals induced by gamma ray treatment showed that the relative concentration of radiation-induced free radicals depends linearly on the absorbed dose.

Research Method

Place and Time

This research was conducted at the experimental field or greenhouse of the Agrotechnology Study Program, University of 17 August 1945 Banyuwangi, from August to September 2025 at an altitude of 24 meters above sea level, the average air temperature was 25.9 ºC and the relative air humidity at the 850mb layer in August - September 2025 ranged from 69% to 86%.

Materials and Tools

The materials used included Prentul F1 chili pepper seeds, planting media consisting of a mixture of cocopeat and rice husk charcoal in a 1:1 ratio, and water for irrigation. The tools used included a Cobalt-60 (^{60}Co) gamma radiation source available at the Radiation Technology Laboratory – Imaging, Irradiation, and Radiation Health Laboratory, Center for Isotopes and Radiation Applications (PAIR), BATAN Jakarta; measuring cylinders; pens and observation sheets; rulers; a camera; and a hoe.

Research Procedure

Seed Preparation and Radiation Treatment

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Prentul F1 chili pepper seeds were prepared according to the required quantity. The seeds were irradiated with the specified doses using a ^{60}Co gamma radiation source at the Center for Isotopes and Radiation Applications (PAIR), BATAN Jakarta.

Seed Germination

The irradiated seeds were sown in a planting medium consisting of a mixture of cocopeat and rice husk charcoal (1:1). The seedlings were maintained by watering every morning and evening.

Research Design and Data Analysis

This study employed a Randomized Completely Block Design (RCBD) with a single factor, namely the dose of ^{60}Co gamma radiation. The treatment consisted of 11 radiation dose levels: 0 Gy (control), 50 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, 350 Gy, 400 Gy, 450 Gy, and 500 Gy. Each treatment was replicated five times, with 10 seeds per replication. The total number of seeds used in the experiment was 11 × 5 × 10 = 550 seeds.

The data obtained were analyzed using analysis of variance (ANOVA) to determine the effect of radiation dose on each parameter. If significant differences were found, further analysis was conducted using Duncan's Multiple Range Test (DMRT) at a 5% significance level. All statistical calculations were performed using the standard statistical software SPSS.

Observations

The parameters observed in this study included:

1. Seed Viability (%)

To calculate seed viability, the most common method is a germination test, which calculates the percentage of successfully germinated seeds from a set number of seeds tested. Data on seed viability percentage were collected 15 days after sowing (DAS). The calculation is done using the following formula:

$$\%\text{Seed Viability} = \frac{\text{Number of seeds that germinated}}{\text{Total number of seeds tested}}\ x\ 100\%$$

2. Seed Mortality (%)

Seed Mortality can be obtained using the formula:

$$\%\text{Seed Mortality} = \frac{\text{Total Number of dead seeds}}{\text{Total number of initial seeds}}\ x\ 100\%$$

3. Mean Germination Time (MGT)

MGT represents the average time required for seeds to germinate. It is calculated using the following formula:

$$\text{MGT} = \frac{\sum(n_i \times t_i)}{\sum n_i}$$

Description:
n_i= number of seeds germinated at observation time t_i

t_i= time (days) at observation

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4. Seedling Height (cm)

The height of Prentul chili seedlings was observed at 15 days after sowing (DAS).

5. Germination Speed Index (GSI)

Data GSI dapat diperoleh dengan menggunakan rumus magure:

$$GSI = \sum \frac{G_i}{T_i}$$

Description:
Gi : Cumulative number of germinated seeds on day *i*

Ti : Day *i* (Observation time)

6. Germination Rate (GR)

Germination rate (GR) is an index that indicates how quickly seeds in a population germinate. The greater the GR value, the faster the germination process. The GR value can be calculated using the following formula:

$$GR = \sum \frac{n_i}{t_i}$$

Description:

n_i = number of seeds germinated on day i

t_i = time (day i)

Results and Discussion

Analysis of variance data showed that Co-60 gamma radiation significantly affected the seed viability, seed mortality, mean germination time (MGT) and seedling height of prentul chili pepper plants. Observations of the average seed viability, seed mortality, mean germination time (MGT) and seedling height of prentul chili pepper are presented in Table 1.

Table 1. Average Seed Viability, Seed Mortality, Mean Germination Time (MGT) and Seedling Height of Chili Seedlings to ^{60}Co gamma radiation

Dose (Gy)	Seed Viability (%)	Seed Mortality (%)	Mean Germination Time (MGT)	Seedling Height (cm)
0	86 a	14 b	10,27 c	3,9 a
50	72 ab	28 ab	10,68 bc	3,8 a
100	60 b	40 a	10,44 c	2,9 b
150	64 ab	36 ab	10,39 c	2,6 bc
200	60 b	40 a	10,44 c	2,2 cd
250	84 ab	16 ab	10,22 c	1,9 d
300	82 ab	18 ab	10,32 c	1,1 e
350	78 ab	22 ab	10,65 bc	1,4 e
400	82 ab	18 ab	11,36 b	1,0 e
450	68 ab	32 ab	13,35 a	1,0 e
500	72 ab	28 ab	12,92 a	1,0 e

The highest average seed viability was observed at the control dose (0 Gy), but was not

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significantly different from other dose treatments, except for the 100 and 200 Gy doses. Meanwhile, the lowest average seed viability was found in the 100 and 200 Gy treatments and was not significantly different from other treatments except for the 0 Gy dose treatment. The physiological response of seeds to gamma radiation is fluctuating and highly dependent on the specific dose received. Low to moderate doses (50–200 Gy) tended to reduce the germination percentage, while at medium doses (250–400 Gy) a stimulative effect was seen that approached the control. Conversely, high doses (> 400 Gy) again reduced seed germination ability.

The results showed that the germination response of chili pepper seeds to ^{60}Co gamma radiation was fluctuating. The significant decrease observed at the 100 Gy dose indicates a strong physiological impact during the early germination stage, possibly caused by DNA damage and disruption of the embryonic cell membrane, which reduced seed viability. This finding is consistent with the report by Marcu et al., (2013), which stated that medium to high radiation doses can damage the cellular and molecular structures of seeds, thereby suppressing their viability potential. Furthermore, at higher doses (250–400 Gy), germination increased again to levels approaching the control, which can be associated with the phenomenon of radio-stimulation.

Gamma radiation within a certain range can trigger self-defense mechanisms such as DNA repair and the activation of antioxidant enzymes, thereby enhancing the seed's ability to grow normally (Shamsiah et al., 2022). However, this positive effect was not consistent, as seen at doses of 450–500 Gy, where the germination percentage decreased again (68–72%). This indicates that the effect of gamma radiation on seed viability greatly depends on the balance between cellular damage and the effectiveness of internal repair mechanisms.

Likewise, the average seed mortality rate showed the highest percentage in the 100 and 200 Gy radiation dose treatments, while the lowest percentage was in the 0 Gy treatment. The seed mortality response showed a fluctuating pattern influenced by variations in radiation dose. At low doses (0–100 Gy), mortality increased from 14% in the control to 28%, then decreased slightly to 26%.

Administration of medium doses (150–200 Gy) caused a sharp increase in mortality, reaching 40% at a dose of 200 Gy. This dose can be considered the optimum dose for mutation induction because it is close to the LD_{50} (50 Gy lethal dose), which is the point at which approximately half of the seed population is unable to survive. In general, doses around the LD_{50} often produce high genetic diversity, because surviving seeds have a greater chance of experiencing mutations (Makhziah & Soedjarwo, 2023). At medium to high doses (250–400 Gy), mortality actually decreased to 16–22%, which was relatively lower than at 200 Gy. Meanwhile, at very high doses (450–500 Gy), mortality rose again to 32%, and then decreased slightly again to 28%.

The highest average data on the Mean Germination Time observation variable was found in the 50 Gy radiation dose treatment but was not significantly different in almost all treatments except for the 450 Gy and 500 Gy treatments. Meanwhile, the lowest average was found in the 450 and 500 Gy radiation dose treatments and was significantly different in all treatments. At low to medium doses (0–300 Gy), the MGT value was relatively stable, ranging from 10.22–10.68 days, and was not significantly different from the control. This condition indicates that gamma radiation at that level has not caused significant physiological damage, so the seed germination rate remains normal. At doses of 350–400 Gy, MGT began to increase (10.65–11.36 days) and showed a significant difference compared to the control. This indicates that at this dose, radiation began to cause sublethal effects in the form of a slowdown in the germination process.

The mechanism of this slowing down is thought to be related to biochemical changes, partial

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DNA damage, or disruption of the enzyme system involved in the initial metabolic processes of germination. At high doses, namely 450–500 Gy, MGT increased sharply to 13.35 days, significantly different from other treatments. The increase in MGT at high doses indicates that gamma radiation has caused serious physiological damage, resulting in seeds requiring longer germination time. High doses of gamma radiation cause physiological changes in the form of delayed germination of cayenne pepper seeds and reduced survival and growth rates. These results are consistent with previous research reporting that the germination potential of wheat seeds decreases with increasing radiation dose (Hong et al., 2022).

Radiation dose treatment affected the height of chili pepper seedlings. Table 1 shows that gamma radiation significantly affected the height of prentul chili pepper seedlings. At control doses (0 Gy) to 50 Gy, seedling height remained relatively high (3.9 cm) and was not significantly different from the control, indicating that radiation exposure at this level did not significantly impact initial plant growth. However, starting at a dose of 100 Gy, seedling height decreased (3.8 cm - 2.9 cm), and this decrease continued consistently as the radiation dose increased.

At doses ranging from 150–250 Gy, seedling height ranged from 2.6–2.2 cm and was significantly different from the control. This indicates that gamma radiation at intermediate doses begins to inhibit vegetative growth due to radiation-induced physiological and metabolic disturbances. A sharp decline occurred at doses of 300-500 Gy, where the seedling height only reached 1.1 cm and was significantly different from all previous treatments (0-250 Gy). This phenomenon indicates a strong growth inhibitory effect, possibly caused by cellular damage, physiological changes, and disruption of enzyme activity that plays a role in cell division and elongation. Marcu et al. (2013) stated that high doses of gamma radiation can inhibit plant growth due to DNA damage, decreased cell viability, and increased free radicals that damage plant tissue.

The Germination Speed Index (GSI) is an indicator that reflects the rate and strength of seed germination per day during the observation period. A higher GSI value indicates faster germination, which means better seed vigor. The data on the effect of ^{60}Co gamma radiation doses on the GSI of Prentul F1 chili pepper seeds are presented in Figure 1.

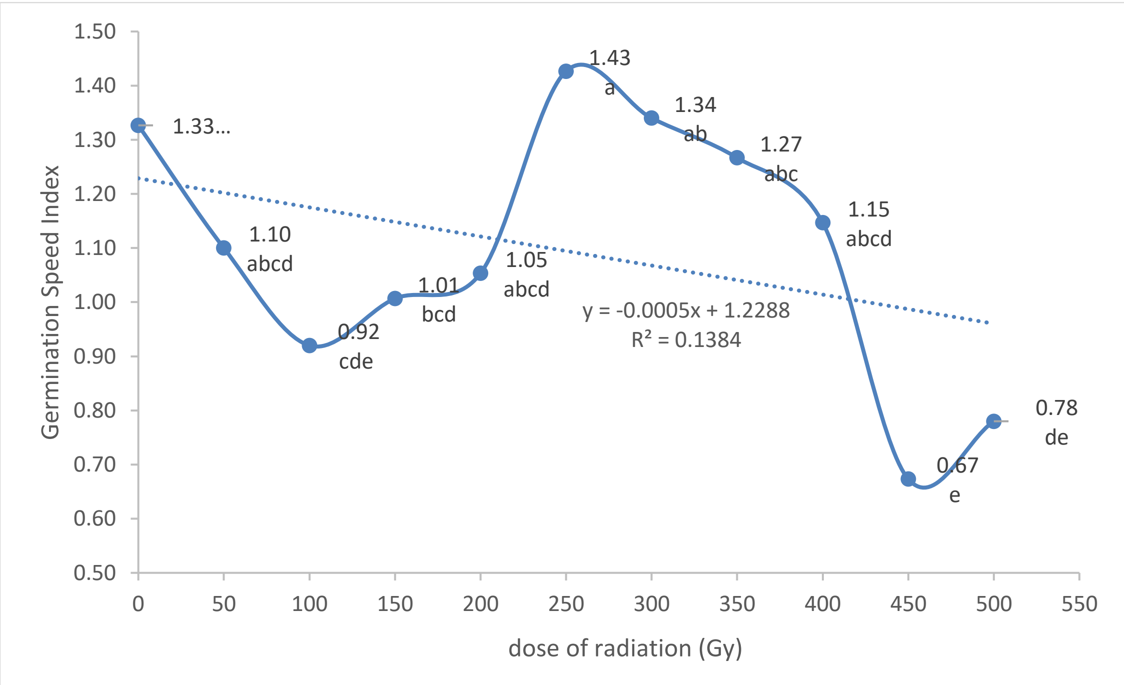

Figure 1. Effect of ^{60}Co Gamma Radiation Dose on the Germination Speed Index (GSI) of Prentul

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Chili Pepper

The data in Figure 1 show that the Germination Speed Index (GSI) of Prentul F1 chili pepper seeds fluctuated with increasing doses of ^{60}Co gamma radiation. At 0 Gy (control), the GSI was around 1.33, then sharply decreased to the lowest value of 0.92 at 100 Gy. Afterward, the GSI increased, reaching its highest peak of 1.43 at 250 Gy, indicating a stimulatory response at this dose. Subsequently, the GSI gradually declined to the lowest point of 0.67 at 450 Gy, before slightly recovering to 0.78 at 500 Gy.

This fluctuating pattern indicates that the effect of ^{60}Co gamma radiation on seed germination speed is non-linear and influenced by the balance between cellular damage and recovery mechanisms. The decrease in GSI at low to moderate doses is likely associated with physiological and molecular damage to seed cells, which inhibits the germination process Marcu et al. (2013). However, the significant increase in GSI at 250 Gy suggests a phenomenon of radio-stimulation, where exposure to radiation at certain doses can stimulate enzymatic activity and metabolic processes that accelerate germination (Shamsiah et al., 2022).

The subsequent decline in GSI at doses above 400 Gy indicates that excessive radiation exposure causes damage exceeding the cellular repair capacity, thereby reducing the seed germination speed. This finding is consistent with the results of Mishra et al. (2024), who explained that plant responses to ionizing radiation are the outcome of complex interactions among oxidative stress, DNA repair, and growth hormone regulation.

The linear regression equation obtained, $y = -0.0005x + 1.2288$ ($R^2 = 0.1384$), indicates a weak negative correlation between radiation dose and GSI, reinforcing that the response pattern does not follow a simple linear trend. Therefore, the effect of gamma radiation on the GSI of chili pepper seeds is strongly influenced by internal physiological mechanisms and specific dose conditions.

The data on the effect of gamma ray (^{60}Co) radiation doses on the average germination time of Prentul chili pepper seeds are presented in Figure 2.

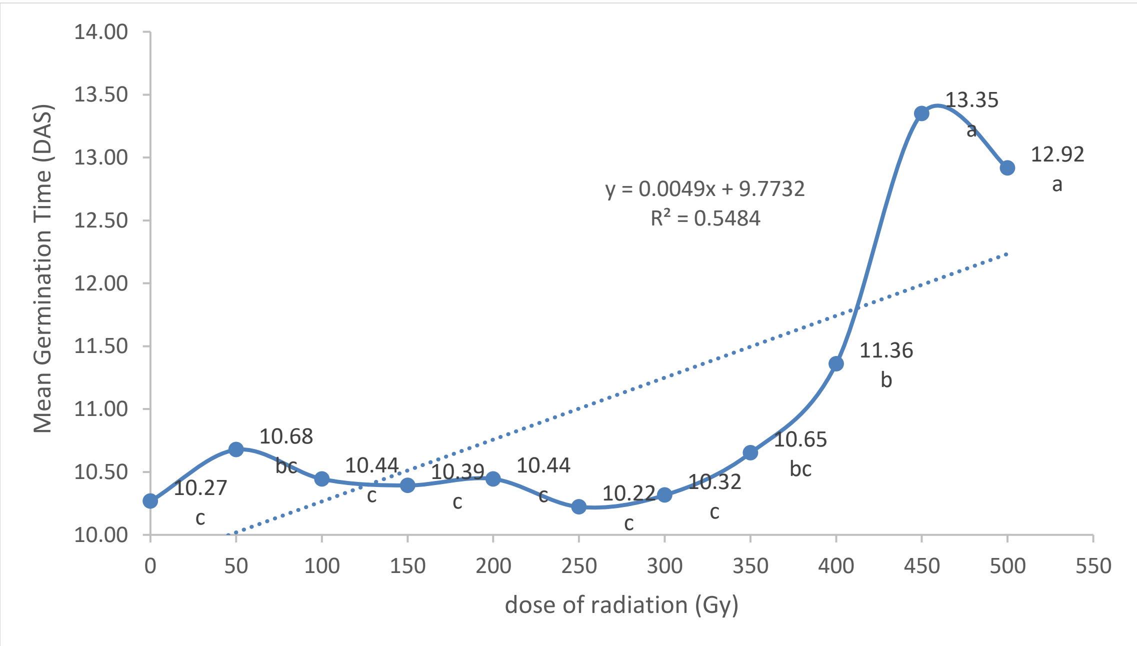

Figure 2. The Effect of Gamma Ray (^{60}Co) Dosage on the Mean Germination Time of Prentul Chili Pepper (*Capsicum frutescens* L.) Seeds

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The results of the study showed that the treatment of gamma radiation doses of ^{60}Co had a significant effect on the average germination time of Prentul chili pepper seeds. Based on the graph in Figure 2, it can be seen that at radiation doses of 0–350 Gy, the average germination time was relatively stable, ranging from 10.22 to 10.65 days after sowing (DAS), with no significant differences among treatments. However, at a radiation dose of 400 Gy, the time required for the seeds to germinate increased to 11.36 DAS, which was not significantly different from the 350 Gy treatment but significantly different from the 450–500 Gy treatments. The germination time of Prentul chili seeds irradiated with higher doses (450–500 Gy) was 13.35 DAS and 12.92 DAS, respectively.

These findings indicate that high-dose Cobalt-60 gamma radiation treatments tend to slow down the germination process of Prentul chili pepper seeds. This delay is presumed to result from metabolic disturbances in the seeds following exposure to ^{60}Co gamma radiation. The results are consistent with previous studies reporting that the germination potential of wheat seeds decreases with increasing radiation doses (Hong et al., 2022). Gamma radiation from ^{60}Co is known to cause damage to embryonic tissues and DNA, thereby slowing seed metabolism and extending germination time (Ling et al., 2008). Moreover, at higher doses, it can lead to increased chromosomal damage, which may reduce germination rate, seedling viability, and plant development Marcu et al. (2013).

In addition, this delay is very likely closely related to the occurrence of oxidative stress within the cells due to the massive formation of free radicals as a response to high radiation exposure. High radiation doses trigger the ionization of water and organic compounds within the cells, producing large amounts of free radicals such as OH^- and H^-. The accumulation of these free radicals can damage cell membranes, proteins, lipids, and genetic material. In addition to slowing cellular physiology, oxidative stress also causes dysfunction in vital organelles such as mitochondria, thereby disrupting the energy (ATP) supply required for seed growth and development (Tias et al., 2022).

Conclusion

The dose of gamma ray radiation significantly affected the variables of average seed viability, seed mortality, average germination time (MGT) and seedling height. In the variable of average seed viability, the highest average was found in the 0 Gy treatment and was not significantly different in all treatments except the 100 and 200 Gy treatments, in the seedling mortality variable, the lowest average mortality was found in the 100 and 200 Gy treatments, but was not significantly different from almost all treatments except the 0 Gy treatment. In the variable of mean germination time (MGT), the fastest average was found in the treatments of 0, 100, 150, 200, 250 and 300 Gy, in the variable of seedling height, the highest average was found in the 0 and 50 Gy treatments and was significantly different in all treatments.

The dose range of 200–300 Gy can be considered as the optimum dose for mutation induction in prentul cayenne pepper.

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