FJARD VOL. 37, NO. 2. PP. 359-373 (2023)

Fayoum Journal of Agricultural Research and Development
ISSN:1110- 7790
On Line ISSN:2805-2528

Impact of using gamma irradiation on germination, growth, yield, and enzymes activity under damping-off disease stress in common bean

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ABSTRACT :

The most dangerous biotic-stress impact in confronting the common bean was damping-off disease. This study aims to examine eco-friendly means instead of using traditional chemical fungicides. Pre-sowing seeds treatment with gradual doses of gamma irradiation (0, 20, 40, 60, 80, and 100 Gy) was chosen compared to Vitavax fungicide. Two germination tests and two open field experiments were conducted during summer seasons of 2021 and 2022 using common bean "Giza-7". Generally, all the treatments were affected significantly all of the studied characters, in both seasons. In particular, the treatments of 20 Gy or Vitavax were significantly enhancing the vigor of common bean plants in confronting the biotic stress of damping-off disease. They, significantly, increased the germination%, survival%, plant height, shoot fresh and dry weights, chlorophyll reading, 100-seed weight, seed yield fad^{-1}, seeds content of N, P, and K%, seeds crude protein, enzymes activity of CAT, POD, and PPO, leaves total phenols and total soluble proteins without significant difference between them. While, the same two previous treatments shared their ability to significantly reduce pre-, post-, and total damping-off percentages, in both seasons. Concerning the cluster analysis of total soluble proteins, the results revealed that the closest treatments were 20 Gy and Vitavax. This study's findings suggest the capability of replacing the fungicide Vitavax with a low dose of gamma irradiation (20 Gy) as pre-sowing seeds treatment for enhancing the physiological status of common bean plants in addition to disease aspects when grown under the biotic stress of damping-off disease.

KEYWORDS: gamma irradiation, biotic stress, damping-off disease, enzymes activity.

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Received: 25/1/ 2023
Accepted: 15/2/ 2023

1- INTRODUCTION:

The common bean (*Phaseolus vulgaris* L.) is one of the most significant edible legumes that cultivated for direct human use globally, especially in African countries where becomes a substantial food in many African countries for its well-known nutritional value as a cheap source of proteins and many nutrients **(Carbas et al. 2020; Didinger et al. 2022)**. The global production of common bean was about 27.5 million tones where Africa shares about 25.7% of the total production of common bean **(FAOSTAT, 2020)**. Therefore, a significant section of researchers is interested in enhancing common bean plants' performance, aiming to increase the yield and quality of the common bean by using eco-friendly means.

Unfortunately, common bean is confronting many types of biotic stresses such as several soil-borne diseases, which resulting in yield losses of up to 80% (Park and Tu 1994). Bean damping-off is one of the most significant diseases caused by a complex of soil-borne pathogens include species of *Fusarium*, *Pythium*, *Rhizoctonia*, and *Macrophomina* that attack beans at all growth stages, causing damping-off at the seedling stage, yellowing of the leaves and declining growth **(Boat et al. 2020; Dignam et al. 2022)**, which ultimately affects the yield. Amongst strategies used to control damping-off, chemical practice is primarily recommended. One of the most important fungicides used in damping-off control is Vitavax, which it also has many physiological effects represented in significant enhancement in germination%, growth, levels of endogenous IAA and GA3, total soluble proteins content, and enzymes activity **(Mohamed and Akladious 2017)**. However, the intensive use of such chemical pesticides on farms could leads to adverse effects on the environment and hazards on humans and livestock, which necessitates the search for issues that are safe on the environment and effective in plant diseases control **(Mahmood et al. 2016)**.

Gamma irradiation is the most energetic form of electromagnetic ionizing irradiation with a very short wavelength, which artificially generated mainly from cobalt-60 **(Bagher 2014)**. Gamma irradiation has been found to be dose-dependent in its effect on plant physiology **(Volkova et al. 2022)**. Also, they mentioned that the plants' response to gamma irradiation is organized by a phenomenon namely hormesis, which is a biphasic dose-response relationship i.e., enhancing growth, accelerated development, improving tolerance to stress factors, or accumulation of compounds of interest in response to low-dose irradiation and *vice versa* with a high dose of gamma irradiation. In addition, the optimal dose of gamma irradiation is considerably varied from 2 to 800 Gy depending on the used plant species, the seed characteristics, and the irradiation features **(Jan et al. 2012; Volkova et al. 2022)**. The effects of gamma irradiation potential should be studied carefully to detect the optimal dose, which widely differs according to the used crop and the propose of application e.g., enhancing growth, mutation induction, alleviating biotic and abiotic stresses **(Piri et al., 2021)**.

Many research papers stated the positive effect of low gamma irradiation doses on germination, growth, endogenous hormonal content, yield, and seeds chemical composition of common bean in addition to enhancing the enzymes activity **(Soliman and Abd-ElHamid, 2003; Mounir et al., 2015a; Mounir et al., 2015b; Bitarishvili et al., 2020; Farid et al. 2021)** According to the results of **Ulukapi (2021)**,the using dose of gamma irradiation (10 or 20 Gy) as common bean seeds treatment was depending on the used cultivar.

Moreover, gamma irradiation seeds treatment was found to be effective in controlling a wide spectrum of abiotic stress causes such as fungi diseases **(Dawar et al., 2010; Said, 2012; Samy et al., 2016; Mardani-Mehrabad et al., 2020)**, viral diseases **(Mardani-Mehrabad et al., 2019)**, nematodes **(Samy et al., 2016)**, and insects **(Hastuti and Sudaryadi, 2020)**.However, the optimal used dose for controlling these infections is varied depending on the plant and the kind of the disease infection.

The aim of this study to explore a suitable dose of gamma irradiation as a pre-sowing treatments of common bean seeds for enhancing plants against damping-off disease, which expressed in germination percentage, vegetative growth, yield, seeds chemical composition, enzymes activity, and phenol content, to be as a double-proposed treatment for enhancing the performance of plants as well as controlling the damping-off disease incidence.

2- MATERIALS AND METHODS:

Two germination tests as well as two field experiments were conducted during summer seasons of 2021-2022at 24/2 and 28/2, respectively. The experiment was carried out in the experimental farm of Etay El-Baroud (Latitude 30° 53' N, Longitude 30° 38' E). The soil texture was categorized as clay (disease analyses of soil if possible). The used cultivar was namely Giza-7.

1. The experimental treatments

Dry seeds of common bean were subjected to seven pre-sowing treatments, five of them consisted of graduated doses of gamma irradiation that were 20, 40,60, 80, 100 Gy in addition to the commercial fungicide "Vitavax" (37.5% Carboxin, 37.5% Thiram) as a comparative treatment, and control treatment that seeds was soaked with tap water. Concerning the gamma irradiation treatments, they have been done using cobalt-60with dose rates of 1.048KGy h^{-1} and 0.873 KGy h^{-1}, in the 2020 and 2021 seasons, respectively at National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Nasr City, Cairo, Egypt.

2. Germination test

Common bean seeds that devoted for germination test was treated with gamma irradiation doses and Vitavax fungicide, in both season of study. Treated seeds were divided onto three replications, 50 seeds for each. The experimental design was Complete Randomized Design (CRD). The procedure and calculation of germination% were conducted as described by **Jussiê et al. (2020)** using the following equation after 7 days from the beginning of the test:

$$\text{Germination\%} = \left(\frac{\text{number of germinated seeds}}{\text{number of initial seeds}}\right) \times 100$$

3. Field experimental design and layout

The followed experimental design for the field experiment was Randomized Complete Block Design (RCBD) with four replicates. Each plot contains five rows with 4 m long and 0.7 m width for each; thus, the plot area was 14 m^2. The common bean seeds were directly sown in the experimental site in two sides of each row alternately, with 15 cm planting distance. All other agricultural practices as soil preparation, fertilization, pest and plant diseases control (except the damping-off disease), and harvesting were done as followed in commercial production of common bean according to the Egyptian Ministry of Agriculture and Land Reclamation.

4. Disease assessment:

Pre- and post- emergence damping-off disease were assessed two and four weeks after sowing, then the survival plants was counted according to the following formula **(Ragab et al. 2009)**.

$$\text{Pre}-\text{emergence}\ (\%) = \frac{\text{Total No. of ungerminated seeds}}{\text{Total No. of planted seeds}} \times 100$$

$$\text{Post}-\text{emergence}\ (\%) = \frac{\text{Total No. of rotted seedlings}}{\text{Total No. of planted seeds}} \times 100$$

Survival plants % = 100 – (pre-emergence + post-emergence damping-off)

5. Field records

The data of vegetative growth traits were recorded using five random chosen plants from each treatment, 60 days after seed sowing. The following measurements were recorded: plant height (cm), shoot fresh weight (g), shoot dry weight (g). at the same stage, the chlorophyll reading were taken using device namely SPAD-502 chlorophyll meter **(Markwell et al. 1995)**.

The collected seeds samples were washed with tap water, distilled water, then oven dried at 70 C° for 48 hours and ground in a mill with stainless steel blades. Wet digestion was performed according to the procedure described by **Chapman and Pratt (1978)**. Then seeds' chemical composition e.g., nitrogen, phosphorus, and potassium as % were determined in the digested solution according to the methods illustrated by **(Temminghoff and Houba, 2004)**. Whereas, the crude protein was calculated by multiplying the N% values by the factor of 6.25. The seed yield fad^{-1} (kg) and 100-seeds (gm) weight were recorded. Seed yield fad^{-1} was calculated by multiplying the seed yield of each plot by the factor of 300 that derived from dividing the faddan area (4200 m^2) on plot area (14 m^2).

6. Leaves' total phenols and enzymes activity

The samples of fresh leaves of common bean plants were taken after 60 days from planting for estimating the total phenols content (mg $g^{-}{}_{1FW}$) as described by **Singleton and Rossi (1965)**; the enzymes' activity (U min^{-1} g^{-1} FW) were determined spectrophotometrically following the method of **Aebi (1984)**for catalase (CAT), the methods of **Amako et al. (1994)**for peroxidase (POD), and the method of **Mayer and Harel (1979)** for polyphenyl oxidase (PPO).

7. Determination of total soluble protein content

Leaf samples of common bean from each treatment were collected at 15 and 30 days from sowing. Three grams of each leaves sample were ground using a mortar in a liquid nitrogen until the sample was completely homogenized. These homogenates were transferred into Eppendorf tubes (1 ml), each containing 200 µl of extraction buffer (50 mM tris-

HCl buffer, pH 6.8, glycerol 10% W/V, ascorbic acid 0.1%, cysteine hydrochloride 0.1% W/V), then centrifuged at 18,000 rpm for one min to remove debris. The protein content in the supernatant was estimated according to **Bradford (1976)** using bovine serum albumin as a standard protein.

8. Cluster analysis of total soluble proteins

Hierarchical Cluster Analysis of total soluble proteins were carried out according to **Baker and Hubert (1975)** using Mstat software program (Version 6.1.4)

9. Statistical Analyses

Data were statistically analyzed using CoStat program. A Least Significant Difference test (LSD) was used at 0.05 confidence level to verify the significance between treatments by using the same program.

3- RESULTS AND DISCUSSION:

1. Germination test

The listed mean values in Table (1) represent the effect of gamma irradiation doses and Vitavax fungicide on germination% of common bean seeds. Generally, the examined treatments showed significant effect on germination% in both seasons. the gamma irradiation dose of 20 Gy followed by Vitavax gave the highest mean values of germination percentage comparing with other treatments where the increase in gamma irradiation doses resulted in decreasing germination%, giving the lowest mean value using gamma irradiation with 100 Gy, in both seasons. these findings could be due to the role of low gamma irradiation (20 Gy) in accelerating the germination process by elevating the endogenous levels of GA3 **(Mounir et al.,2015a; Mounir et al., 2015b; Volkova et al., 2022)**. In this respect, **Bitarishvili et al. (2020)** suggested that 20 Gy gamma irradiation treatment increased the expression of gibberellin biosynthesis on barely. Also, **(Mohamed and Akladious, 2017)** found that Vitavax resulted in increasing germination% and endogenous levels of IAA and GA3 in cotton seedlings.

Table 1. Mean values of common bean seeds germination% as affected by gamma irradiation doses andVitavax treatments at 2021 and 2022 seasons.

Pre-sowing seeds treatments	Germination%*	
	2021	2022
control	93.67 bc	94.33 bc
20 Gy	96.67 a	97.00 a
40 Gy	93.00 c	94.00 cd
60 Gy	92.67 cd	93.67 cd
80 Gy	91.00 d	92.33 d
100 Gy	88.00 e	89.00 e
Vitavax	95.00 ab	96.00 ab

*** Means with the same letter(s) do not differ significantly under 0.05 confidence level**

2. Disease assessment

Mean values in Table (2) express the effect of gamma irradiation doses and Vitavax fungicide on damping-off disease parameters in 2021 and 2022 seasons. the treatments were found to affect significantly the pre, post, total damping-off, and survival% of common bean seedlings under natural infection field conditions, in both seasons. The control and gamma dose of 100 Gy treatments gave the highest mean values of pre, post, total damping-off%, and survival%; while, treating common bean seeds with Vitavax or 20 Gy of gamma rays had the lowest significant mean values of pre, post, total damping-off %, and survival% without significant differences between them, in both seasons.

These results could be a result of increasing of gibberellins that accelerate the germination process, which reflects on shorting the probability of infection with one of damping-off pathogens where represented in this study in significant reduction in mean values of pre, post, and total damping-off as well as increasing the survival%, in both seasons. These effects of gamma irradiation seed treatments on damping-off disease control were reported by **Dawar et al. (2010)** on sunflower and mung bean, **Ikram et al. (2010)** on mung bean, **Samy et al. (2016)** on soybean, which were causing decrease damping-off indications. Also **Said (2012)** found that the chickpea seeds gamma irradiation treatments were significant in control root rot disease, which decreased the pre-emergence infected plants% that reflect on increasing the survival plants (%). Moreover, **Helal (2017)** found that the treating of squash seeds with 20 Gy gamma irradiation was resulted in decreasing the percentages of pre- and post-emergence damping-off and increasing the survival% when sowned in infested soil with *Rhizoctonia solani* comparing with control. This effect of gamma irradiation on damping-off biotic stress may attributed to the reduction in microbial load of the treated common bean seeds surface **(Lima et al., 2019)** that is in favor of lower damping-off infection rates.

Table 2. Mean values of pre-, post-, total damping off, and survival percentages as affected by gamma irradiation doses and Vitavax treatments at 2021 and 2022 seasons.

Pre-sowing seeds treatments	Pre- damping off%		Post- damping off%		Total damping-off%		Survival%	
	2021	2022	2021	2022	2021	2022	2021	2022
control	23.00a	24.33a	24.67a	25.00a	47.67a	49.33a	52.33f	50.67f
20 Gy	10.00e	12.33 d	11.67d	13.00e	21.67e	26.00d	78.33a	74.00a
40 Gy	14.67d	16.33c	14.00c	16.33d	28.67d	32.67c	71.33b	67.33b
60 Gy	17.33c	17.67bc	15.67c	18.00cd	33.00c	35.67c	67.00c	64.33c
80 Gy	19.00b	20.00b	18.33b	20.67bc	37.33b	40.67b	62.67d	59.33d
100 Gy	23.00a	23.33a	22.67a	23.00ab	45.67a	46.33a	54.33e	53.67e
Vitavax	10.33e	13.33d	11.33d	12.67e	21.67e	25.33d	78.33a	74.67a

* Means with the same letter(s) do not differ significantly under 0.05 confidence level

3. Vegetative growth and chlorophyll reading

The effect of seeds treatments with gamma irradiation doses and Vitavax on plant height, shoot fresh and dry weights mean values were listed in Table (3) in addition to chlorophyll readings that illustrated in Fig. (1). The used treatments were found to be significantly affected the measured characters, in both seasons. Also, the gamma irradiation treatment of 20 Gy followed by using Vitavax fungicide were significantly effective in increasing mean values of previously mentioned traits without significant difference between them, in both seasons. Otherwise, the increasing gamma irradiation doses were found to decrease the mean values of above mentioned characters, in both seasons. These findings were in line with **(Soliman and Abd-ElHamid, 2003; Mounir et al., 2015a; Mounir et al., 2015b; Mounir et al., 2022)** who stated the positive hormetic effect of low gamma irradiation (mostly 20 Gy) that reflect on the growth parameters as well as the chlorophyll. Also, **Ulukapi (2021)** stated that the using of low-dose gamma irradiation (20 Gy) accelerates cell division, which explains the increase in plant growth. The enhancement of vegetative growth characters and chlorophyll content due to pre-sowing seeds treatment with low gamma irradiation dose (20 Gy) could be due to its induction of endogenous levels of plant hormones e.g., GA3 and IAA **(Bitarishvili et al., 2020; Mounir et al., 2022 and Volkova et al., 2022)**.

Table 3. Mean values of plant height, shoot fresh and dry weights, and chlorophyll as affected by gamma irradiation doses and Vitavax treatments at 2021 and 2022 seasons.

Pre-sowing seeds treatments	Plant height (cm)		Shoot fresh weight (g)		Shoot dry weight (g)	
	2021	2022	2021	2022	2021	2022
control	43.4d	43.00cd	21.73d	24.10de	5.24bc	5.25cd
20 Gy	50.6a	50.70a	24.03a	27.97a	6.00a	6.03a
40 Gy	46.93bc	46.03b	22.53bc	25.80bc	5.48b	5.57bc
60 Gy	45.70cd	44.23bc	22.07cd	24.40cd	5.29bc	5.43c
80 Gy	38.63e	40.70de	19.57e	22.77ef	4.92cd	4.93de
100 Gy	34.93f	39.67e	18.93e	22.40f	4.68d	4.74e
Vitavax	48.93ab	50.30a	22.90b	27.23ab	5.94a	5.97ab

* Means with the same letter(s) do not differ significantly under 0.05 confidence level

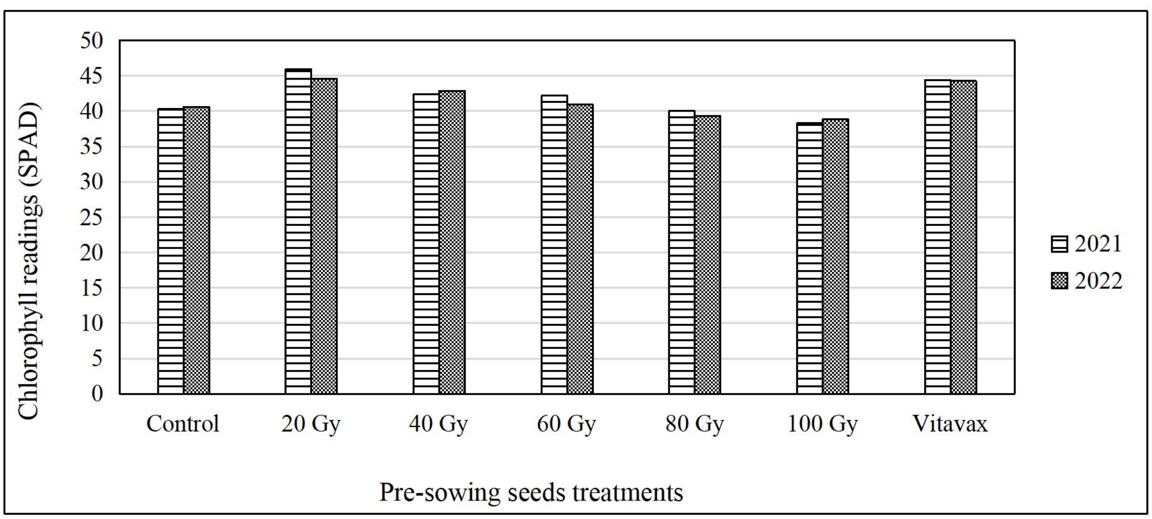


Figure 1. Effect of pre-sowing seeds gamma irradiation doses and Vitavax treatments on chlorophyll readings (SPAD) of common bean leaves at 2021 and 2022 seasons.

4. Yield and 100-seeds weight

The showed mean values in Table (4) exhibit the effect of gamma irradiation and Vitavax on yield and 100-seeds weight of common bean. The results revealed that, application of 20 Gy gave the highest mean values of seed yield fad^{-1} and 100-seeds weight comparing with other treatments followed by Vitavax treatment without significant difference between them, in both seasons. In contrast, the using of 100 Gy as a pre-sowing treatment was give the lowest mean values of seed yield fad^{-1} and 100-seeds weight, in both seasons. These findings were in harmony with those of **Mounir et al. (2015a); Mounir et al. (2015b)** how reported that the pre-sowing seeds low gamma irradiation as 20 Gy was significantly increase the total yield of snap bean.

Table 4. Mean values of 100-seed weight, and seed yield fad^{-1}as affected by gamma radiation doses and Vitavaxseed treatments at 2021 and 2022 seasons.

Pre-sowing seeds treatments	100 seed weight (gm)		Seed yield fad^{-1} (kg)	
	2021	2022	2021	2022
control	40.45de	37.02de	602.75de	572.95de
20 Gy	46.64a	47.50a	675.56a	675.55a
40 Gy	41.24bc	43.38bc	634.95bc	623.82bc
60 Gy	41.35cd	39.50cd	612.01cd	593.21cd
80 Gy	38.34ef	33.10ef	584.33ef	541.23ef
100 Gy	37.03f	31.67f	559.41f	541.23ef
Vitavax	44.60ab	43.60ab	662.53ab	648.14ab

* Means with the same letter(s) do not differ significantly under 0.05 confidence level

5. Seeds chemical composition

The mean values showed in Table (5) represent the effect of gamma irradiation doses and Vitavax on the common bean seeds content of N, P, K%, and crude protein contents during 2021 and 2022 seasons. generally, the applied treatments were showed significant effect on such characters, in both seasons. Also, the results showed that treatments either gamma rays at 20 Gy or Vitavax fungicide gave the highest significant common bean seeds chemical N, P, K% and crude protein compared with other examined treatments, in both seasons. the findings could be due to the positive effect of pre-sowing treatment with eco-friendly low gamma radiation that activate the physiological status of common bean plants that started from germination until producing yield with high N, P, and K content as well as high crude protein content. Therefore, the pre-sowing seeds treatment with 20 Gy gamma irradiation could result in significant increase in protein content of snap bean **(Mounir et al., 2015b).** Also, **(Moreira et al., 2021)** found that the treated seeds with 20 Gy of gamma rays gave the highest crude protein content in produced soybean seeds.

Table 5. Mean values of N%, P%, K% and crude protein contents of produced seedsas affected by gamma irradiation doses and Vitavax seed treatments at 2021 and 2022 seasons.

Pre-sowing seeds treatments	N%		P%		K%		Crude protein	
	2021	2022	2021	2022	2021	2022	2021	2022
control	3.27bc	3.18b	0.349bc	0.352bc	2.53ab	2.55ab	20.44bc	19.85b
20 Gy	3.41a	3.39a	0.410a	0.380a	2.64a	2.59a	21.33a	21.21a
40 Gy	3.26bc	3.14bc	0.336bc	0.336cd	2.47bc	2.51bc	20.35bc	19.63bc
60 Gy	3.16cd	3.01cd	0.319c	0.337c	2.39cd	2.48c	19.73cd	18.83cd
80 Gy	3.08de	2.98d	0.279d	0.311de	2.29de	2.39d	19.27de	18.63d
100 Gy	3.00e	2.69e	0.275d	0.229e	2.27e	2.36d	18.73e	16.83e
Vitavax	3.31ab	3.34a	0.370ab	0.377ab	2.60a	2.55a	20.71ab	20.88a

* Means with the same letter(s) do not differ significantly under 0.05 confidence level

6. Leaves enzymes activity and total phenols content

The effect of gamma irradiation doses and Vitavax fungicide on enzymes activity and total phenols content of common bean leaves in Table (6) revealed significant effect in both seasons. Also, the represented mean values showed that the treated common bean seeds with 20 Gy gamma irradiation or Vitavax fungicide gave the highest mean values of enzymes activity of CAT, POD, PPO, and total phenols without significant difference between them. In contrast the corresponding mean values under 100 Gy were significantly the lowest, in both seasons. Moreover, the comparison between the mean values of 20 Gy gamma irradiation and their corresponding mean values in control revealed that the CAT enzyme activity was the most responded enzyme with increment percentage of 24.15% followed by PPO, total phenols, and POD, which gave 18.91%, 12.55%, and 6.24%, respectively, as a mean of both seasons increment percentages. These findings were found to in agreement with those of **Mardani-Mehrabad et al. (2020)** who found the gamma irradiation doses of 20-30 Gy were significantly effective in inducing the enzyme activity such as CAT and PPO in common bean; also, on barley, **Hussein (2022)** stated that the gamma irradiation dose of 20 Gy gave the highest enzymes activity of CAT and POD on barely plants. Also, **Maraei and Elsawy (2017)** reported a positive effect of gamma irradiation on the phenolic content in strawberry plants. In addition, the results of **Hanafy and Akladious (2018)** revealed that the 25 Gy gamma irradiation as fenugreek seeds treatment resulted in a significant increase in total phenol content compared with the control. Also, **Mounir et al. (2015b)** reported a significant increase in total phenols in snap bean leaves compared with control as a result of pre-sowing seeds treatment with 20 Gy of gamma irradiation. Moreover, **(Mohamed and Akladious, 2017)** on cotton seedling, found that the seeds treatments with Vitavax resulted in enhancement in enzymes activity of POD and CAT.

Table 6. Mean values of CAT, POD, PPO and total phenols in common bean leaves as affected by gamma irradiation doses and Vitavax seed treatments at 2021 and 2022 seasons.

Pre-sowing seeds treatments	CAT ($U\ g^{-1}$ FW)		POD ($U\ g^{-1}$ FW)		PPO ($U\ g^{-1}$ FW)		Total phenols ($mg.g^{-1}$ FW)	
	2021	2022	2021	2022	2021	2022	2021	2022
control	0.300cd	0.340de	0.643cd	0.713cd	0.367d	0.360cd	3.45cd	4.04cd
20 Gy	0.427a	0.430a	0.783a	0.800a	0.510a	0.517a	4.99a	4.82a
40 Gy	0.343b	0.397bc	0.743b	0.747bc	0.440bc	0.413b	4.32b	4.47b
60 Gy	0.323bc	0.367cd	0.673c	0.720cd	0.393cd	0.387bc	3.90bc	4.21c
80 Gy	0.27d	0.337de	0.630de	0.703de	0.350de	0.242d	3.17de	3.95de
100 Gy	0.267d	0.323e	0.597e	0.663e	0.313e	0.337d	2.86e	3.72e
Vitavax	0.423a	0.420ab	0.763ab	0.780ab	0.467ab	0.490a	4.80a	4.72a

* Means with the same letter(s) do not differ significantly under 0.05 confidence level

7. Leaves total soluble proteins

The mean values of leave content of total soluble protein content of common bean leaves, which its seeds previously were treated with augmented doses of gamma irradiation (0, 20, 40, 60, 80, and 100 Gy) as well as Vitavax fungicide were listed in Table (7). Generally, the examined treatments affected significantly the leaves total soluble protein, in both seasons.

The treatments of 20 Gy and Vitavax fungicide gave significantly the highest mean values of leaves total soluble proteins compared with the other treatments at the two sampling dates (15 and 30 days from sowing date), in both seasons. In the case of the 20 Gy irradiation dose, it was found to give a higher mean value of total soluble proteins estimated as 24.55% for 15 days from sowing and 19.97% for 30 days from sowing, as averages of the two seasons of study.

Total soluble proteins were declared as a reliable indicator for plant defense status against different diseases stress **(Goicoechea et al., 2000; Hameed et al., 2008; Mohamed et al., 2018)**. **Yasmin et al. (2019)** declared that several proteins were synthesized and accumulated in plant tissues under a wide range of stress conditions.

The findings of this study are in harmony with those of **Desai and Rao (2014)**, which illustrate that the total soluble content protein content of the irradiated pigeon pea seeds shown some differences depending on the gamma irradiated doses, which the low gamma irradiation dose resulted in high total soluble protein and *vice versa* concerning the high doses. The results of **Moussa (2011)** revealed a significant effect of 20 Gy as a pre-sowing treatment of soybean seeds on increasing the plant vigor grown under stress where it was illustrated with a significant increase in total soluble proteins comparing with control. Moreover, **Hussein (2022)** found that exposure of the seeds to low doses of gamma irradiation (0, 5, 10, and 20 Gy) induced the synthesis of new proteins in barley plants. In addition, **(Mohamed and Akladious, 2017)** stated that seeds treatment with Vitavax resulted in increasing the total soluble proteins content of the cotton seedlings. Moreover, The depletion of some proteins may be due to higher metabolic and hydrolyzing enzyme activities **(Maity et al., 2004)** and/or to disturbance of the protein synthesis leading to incorrect folding and damage protein structure **(Hameed et al., 2008)**.

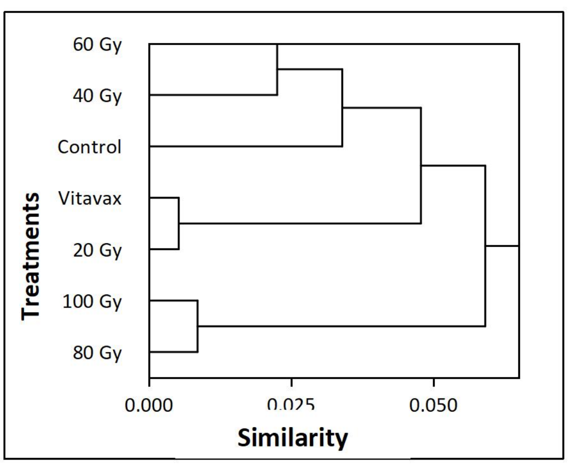
Table 7. Mean values of total soluble protein in common bean leaves as affected by gamma irradiation doses and Vitavax seed treatments at 2021 and 2022 seasons.

Pre-sowing seeds treatments	15 days from sowing		30 days from sowing	
	2020	2021	2020	2021
Control	24.75 c	23.33 c	35.20 c	30.31bc
20 Gy	30.63 a	29.24 a	39.86 a	38.40 a
40 Gy	27.49 b	26.34 b	36.34 c	36.23 a
60 Gy	26.90 b	24.56bc	35.28 c	31.51 b
80 Gy	22.59 d	20.12 d	33.74 d	28.63 cd
100 Gy	22.41 d	19.58 d	33.35 d	27.56 d
Vitavax	30.34 a	28.90 a	38.35 b	37.31 a

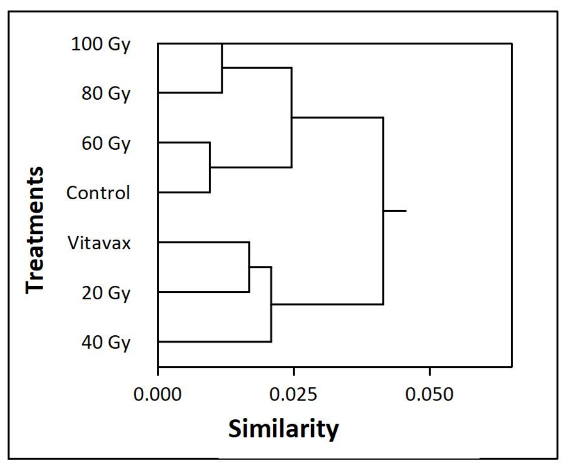
* Means with the same letter(s) do not differ significantly under 0.05 confidence level

8. Cluster analysis of total soluble proteins

The cluster analysis for similarity between total protein content through different Gy levels and Vitavax treatments is illustrated in Fig. (1). The different treatments were clustered in distinct groups in the bases of similarity among the different treatments during the two sampling dates (15 and 30 days from sowing). Firstly, in the first date (15 days), the examined treatments were divided onto two major groups; the first group was consisted of 40 Gy, 60 Gy, control, Vitavax, and 20 Gy. The first group, in turn, divided onto three sub groups that revealed high similarity between each of 40 Gy and 60 Gy treatments and between Vitavax and 20 Gy treatments. Concerning the second major group, it was found to consist of 80 Gy and 100 Gy treatments. After 30 days from sowing, the similarity test produced two major clusters. The first consists of two sub-groups (Vitavax, and 20 Gy; and 40 Gy). The second major group was found to consist of two sub-groups, which were 80 Gy and 100 Gy treatments; and 60 Gy and control. These findings revealed that each of 20 Gy and Vitavax pre-sowing seeds treatment has the same effect on the total soluble proteins in common bean leaves.



15 days from sowing



30 days from sowing

Fig 2. Dendrogram of cluster analysis for total soluble proteins means similarity under different gamma irradiation doses, Vitavax and control treatments.

CONCLUSION:

The results of this investigation could be conclude that the ability of replacing Vitavax chemical fungicide with the eco-friendly treatment of gamma irradiation at the dose of 20 Gy as a pre-sowing seeds treatment for confronting the biotic stress of damping-off disease stress as well as enhancing growth and yield of common bean plant cv. Giza-7.

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الملخص العربي

تأثير استخدام إشعاع جاما على الإنبات ، والنمو ، والمحصول ، ونشاط الإنزيمات في ظل إجهاد مرض الذبول على االفاصوليا

يُعَد مرض الذبول من أكثر الإجهادات الحيوية خطورة و التي تواجهة الفاصوليا. تهدف هذه الدراسة إلى دراسة وسيلة صديقة للبيئة بدلاً من استخدام مبيدات الفطريات الكيميائية التقليدية. تم اختبار معاملة البذور قبل الزراعة بجرعات تدريجية من أشعة جاما (0 ، 20 ، 40 ، 60 ، 80 ، 100 جراي) و مقارنتها مع المبيد الفطري فيتافاكس. تم إجراء اختبارين للإنبات وتجربتين حقليتين في موسمي 2021 و 2022 باستخدام الفاصوليا صنف "جيزة 7". بشكل عام فإن جميع المعاملات التي تم اختبارها قد أثرت معنوياً في جميع الصفات المدروسة في كلا الموسمين. على وجه الخصوص ، كانت معاملات 20 جراي أو الفيتافاكس هي الأكثر فاعلية في تعزيز قوة الفاصوليا في مواجهة الإجهاد الحيوي لمرض الذبول. لقد أدتت لك المعاملات إلى زيادة معنوية في نسبة الإنبات ونسبة البقاء على قيد الحياة وارتفاع النبات (سم) وأوزان المجموع الخضريالطازجة والجافة (جم) والكلوروفيل (سباد) ووزن 100 بذرة (جم) ومحصول البذور (كجم للفدان) محتوى البذور من النتروجين و الفوسفور و البوتاسيوم والبروتين الخام ونشاط إنزيمات الكاتاليز و البيروكسيديز و البولي فينايل أوكسيديزوالفينولات الكلية والبروتينات الذائبة الكلية بالأوراق دون اختلاف معنوي بينهما. على العكس من ذلك، فإن تلك المعاملتين السابقتين تشتركان في قدرتهما على تقليل نسب الذبول الكلية قبل وبعد الإنبات ، بشكل معنوي ، في كلا الموسمين. فيما يتعلق بالتحليل العنقودي للبروتينات الذائبة الكلية ، أوضحت النتائج أن أقرب العلاجات كانت 20 جراي و الفيتافاكس .تشير نتائج هذه الدراسة إلى إمكانية استبدال مبيد الفطريات الكيميائي فيتافاكس، باستخدام جرعة منخفضة من أشعة جاما (20جراي) كمعاملة للبذور قبل الزراعةلتحسين الحالة الفسيولوجية لنباتات الفاصولياخاصة عند نموها تحت ظروف الإجهاد الحيوي لمرض الذبول.

الكلمات الدالة: الفاصوليا، أشعة جاما، الإجهاد الحيوي، مرض الذبول، النشاط الإنزيمي