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USING BIOTIC AS ALTERNATIVES TO CONTROL POWDERY MILDEW IN IRRADIATED CUCUMBER SEEDS

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ABSTRACT

Inducing resistance against powdery mildew caused by *Sphaerotheca fuliginea* in cucumber (cv. Beta Alfa) was investigated under filed conditions as fungicides alternatives. Biotic inducers (*Bacillus subtilis*) were sprayed onto the upper two true cucumber leaves. And water was used as negative control. Further, specific activities of defense-related enzymes (peroxidase, polyphenol-oxidase,catalses) and total phenols were spectrophotometrically measured in cucumber leaves and after inoculation with fungal spores. Results showed that, biotic tested inducers reduced the disease severity on the treated leaves, increased (plant length, root length, and plant dry weight) and increased the activities of defense-related enzymes, low doses of gamma radiation increased the vegetative growth specially doses at 15 and 20 Gray (Gy).

Key words: Cucumber, powdery mildew, *Sphaerotheca fuliginea*, *Bacillus subtilis*, gamma radiation, peroxidase activity, poly phenol oxidase, catalse activity, total phenols.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the most important economic vegetable crops of those belong to family Cucurbitaceae. The economic importance of this crop appears in both local consumption and exportation purposes. Cucumber is grown either in the open field or under protected houses (**Hanam *et al.,* 1978**).

Egypt is among top ten cucumber producing countries **FAO STAT data (2012).** Powdery mildew is a common disease of both domesticated and wild species of cucurbits; it is one of the most destructive leaf diseases, affecting mainly cucumber (*Cucumis sativus* L.) among the several cucurbits **(Zatarin *et al.*, 2005)**. Biological control of disease using biological agent such as *Tirchoderma*. spp and *Bacillus subtilis* for controlling foliar pathogenic fungi has been recorded by many researchers **(Elad, 2000; Abd El-Moneim, 2004; Mahdy *et al*, 2006).**

In Egypt**, Ahmed (1995)** mentioned that powdery mildew disease on cucumber was caused by *Sphaerotheca fuliginea* not *E. cichoracearum*. **Mosa (1997)** indicated that Powdery mildew of cucumber is one of the most important foliar diseases attacking cucumber plant in Egypt and other countries. **Abd-El-Sayed (2000)** reported that the casual agent of powdery mildew on cucumber in Egypt was *Sphaerotheca fuliginea*, not *E. cichoracearum*. **Meliki and Marouani (2010)** concluded that irradiated and not irradiated wheat seed showed no significant differences at low dose.

Borzouei *et al.* (2010) results showed that a radiation dose of 15 Gy caused

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improvements in chickpea dry weight in contrast to 0 Gy doses of gamma rays. This study aimed to use biotic as alternative safe methods to control cucumber powdery mildew.

MATERIALS AND METHODS

Two filed experiments were carried out during growing seasons 2013-2014 and 2014-2015 at experimental farm, Department of Plant Research, Nuclear Research Center (NRC), Atomic Energy Authority, Inshas, Egypt.

Plant Materials

Cucumber seeds (*Cucumis sativus* L.) cv. (Beita Alpha F.1), obtained from Agriculture Research Center, Giza, Egypt, were sown on August and September of 2013 and 2014, growing seasons respectively.

Irradiation of Gamma Rays

Dry seeds of cucumber were kept in small plastic trays (100 g. each) and exposed to Gamma rays (Co^{60}) at 5-doses, 0, 10, 15, 20, and 25 Gray (Gy). irradiation took place at Nuclear Research Center, Inshas, Egypt.

Source of Microorganisms

Source of *Sphaerotheca fuliginea* conidia

Conidia were harvested, according to **Nair *et al.* (1962)**, from young cucumber leaves which showed extensive powdery mildew symptoms.

Old conidia were omitted and the newly ones were used in the laboratory trials.

Source of *Bacillus subtills*

Bacillus subtills bacteria were obtained from (Mersin center) Ain Shams University, Egypt. Concentrations used $10x10^{10}$ and $10x10^{12}$ Cfu/ml.

Disease assessment

Disease assessment was conducted at 12 days after inoculation (field conditions). Inoculated plants were carefully examined to estimate the severity of infection by powdery mildew disease as affected by the different tested treatments was depending on the devised scale (0-4) by **(Reuveni *et al.*, 2000)** using the following formula:

Disease severity (%) = $(\Sigma$ (n x v) /5N x 100.

Where n = number of infected leaves in each category; v = numerical values of each category; N = total number of the infected leaves.

Inoculation

Cucumber leaves were carried out from Ain Sahms University, Faculty of Agriculture, Egypt and kept in paper pages. The cucumber host was grown in field (field conditions followed), and at first two true leaves expansion, the infected leaves were allowed to remain on the plants by leaf contact and indirect transfer was made. Then the heavy infected were collected and the spore suspension was poured into a volumetric flask. The flask was shaken for a few minutes before counting the spores by haemocytometre. The spore suspension was adjusted to a concentration of 10.32x10 spores per ml.

Enzymes assay

Peroxidase activity was measured by a modified method with guaiacol **(Angelini *et al.* 1990).** The activity of Poly phenol oxidase was determined according to the method of **(Esterbauer *et al.*, 1977)**. Catalase activity was determined with spectro-photometric method at 240 nm according to **(Tkhemaladze and Kvesitadze, 1975).** Total Phenols were determined by using Folin- Ciocalteu method with catechol according to **(Singleton and Rossi, 1965)**.

Statistic analysis

The Data for both seasons were statistically analyzed using split plot design analysis according to **(Snedecor and Cochran, 1982**) multiple range test at 0.05 levels.

RESULTS AND DISCUSION

Effect of Certain Doses of Gamma Irradiation on Plant Morphological Characteristics

First season trial (2013)

Data in Table 1 showed that the tested gamma doses except the dose at 25 Gray had significant impact on the morphological characters (plant length, root length, leaf length, and leaf width and plant dry weight) of treated cucumber plants infected with powdery mildew as compared with untreated (0 Gy) after 40days of planting. The highest plant length, root length, leaf length, leaf width and plant dry weight (34 cm, 6.5 cm, 6.3 cm, 6.7 cm and 3.7 g) detected with 20 Gy as compared with control treatment *i.e.,* untreated (0Gy) (28.3 cm, 4.2cm, 5 cm, 4.5 cm and 2.7g), the highest tested gamma irradiation dose (25 Gy) had detrimental effect on plant growth (29.7cm, 4.7cm, 5.5 cm, 4.7 cm and 2.8g).

Second season trial (2014)

In the second season trial (2014), the obtained results showed the same trend. All teased gamma doses significantly increase (plant length, root length, leaf length, leaf width, plant dry weight) as compared with control treatment un irradiated or (0Gy) except dose at 25 Gy.

The highest plant length, root length, leaf length, leaf width and plant dry weight (37.7cm, 7cm, 6.7cm, 6.8 cm and 3.7g) were detected with 20Gy as compared with control treatment (untreated or without radiation 0Gy) (30.3cm,5.2cm,5.2cm,2.5cm and 2.9g). While the highest tested gamma radiation dose (25Gy) also had detrimental effect of the tested plant characteristics (30.3cm, 5cm, 5.7cm, 5 cm and 3.1g)

Statistically there were significant differences in the morphological characteristic among tested gamma doses and control treatment (0Gy) expect dose at 25 Gy.

In general, all growth parameters were increased gradually with increasing doses of gamma rays up to 15 and 20 Gy irradiated seeds with 15 and 20 Gy seems to be the best doses for increasing all vegetative growth parameters these results are in harmony with those of **(Veeresh *et al.*, 1995)** studied on dry seeds of been, exposed to different gamma ray doses to study their effect on various plant characters like germination, shoot length, root length, fresh and dry weight of plants. The results showed that there was more reduction at higher doses compared to lower doses for all the characters.

Muhkin *et al.* (1979) stated that the greatest stimulation effect during presuming gamma irradiations are established when cabbage seeds treated with doses vary from 3 to 8 Gy on the other hand, the results of vegetative growth seemed to decrease with increasing of radiation doses.

Kim *et al.* (2001) investigated that the effect of low doses of gamma radiation on the field growth parameters 45 days after planting of mustered seeds noticeably at the entire low dose irradiation group.

Effect of *Bacillus subtilis* and Certain Doses of Gamma Irradiation on Disease Severity

Garg *et al.* (1972) found that Irradiated barley seeds with 15 and 20 Gy, seems to be the best doses for increasing all vegetative growth parameters such increments in vegetative growth could be referred to the irradiation improved plant growth, maintained an active metabolism in plants, cytological and physiological changes at cellular level inducted by gamma radiation.

In the first season trial (2013), the highest plant length was observed with *B.subtilis* $10x10^{10}$ and $10x10^{12}$ (30.3 and 31.7cm) as compared with control untreated (28.3cm), for root length 5.3 and 6cm as compared with control *i.e.* untreated 5cm, for plant dry weight 3.5g and 3.6 g as compared with control untreated 2.7 g.

Table (1): Effect of certain doses of gamma irradiation on plant morphological characteristics in cucumber plant.

Radiation (Gy)	Plant morphological characters					
	Plant length (cm)		Root length (cm)		Plant dry weight (g)	
	Seas.1	Seas.2	Seas.1	Seas.2	Seas.1	Seas.2
0	28.3h	30.3j	4.2g	5.2f	2.7k	2.9i
10	30.7ef	33gh	5.2e	5.8e	3i	3.3g
15	32.7de	35.7e	6.2bc	6.3de	3.2h	3.5f
20	34c	37.7c	6.5b	7bc	3.7g	3.7e
25	29.7g	30.3j	4.7f	5f	2.8k	3.1h

Means in the same column followed by different letters indicate significant difference (LSD, P>0.05)

Table (2-a): Effect of *Bacillus subtilis* and certain doses of gamma radiation on disease severity during second season of 2013.

Gamma radiation (Gy)	Diseases severity (%) season (2013)		
	Control	***Bacillus subtilis* (cfu/ml)**	
		$10x10^{10}$	$10x10^{12}$
0	38.80a	18.60b	19.30b
10	38.40a	18.40cd	18.33de
15	38.60a	18.20c	18.50d
20	38.60a	18.60b	18.60cd
25	38.60a	18.60b	18.90c

Means in the same column followed by different letters indicate significant difference (LSD, P>0.05).

Table (2-b): Effect of *Bacillus subtilis* and certain doses of gamma radiation on disease severity during second season of 2014.

Gamma radiation (Gy)	Diseases severity (%) season (2014)		
	Control	***Bacillus subtilis* (cfu/ml)**	
		$10x10^{10}$	$10x10^{12}$
0	37.60a	19.13b	19.30b
10	37.33a	18.73c	18.80c
15	37.33a	18.50cd	18.63cd
20	37.33a	18.30d	18.03d
25	37.40a	19.03bc	19.20bc

Means in the same column followed by different letters indicate significant difference (LSD, P>0.05).

In the second season the same trend was observed. All treatments significantly increased morphological characteristics the highest plant length was observed with *B. subtilis* $10x10^{10}$ and $10x10^{12}$ (31.3 and 31.3cm) as compared with control untreated 30.3 cm, for root length the value were 5.5 and 6.2cm as compared with control i.e. untreated 5.2cm, for plant dry weight 3.6g and 3.7 g as compared with control untreated 2.9 g.

Statistically, there were significant differences in the morphological characters among tested treatments and control treatment (untreated) in first and second season trial.

These results are in agreement with **Compants *et al.* (2005)** who indicated that *Bacillus subtilis* not only antagonized several pathogenic fungi but also promoted the seedling growth and increased its stress tolerance. Regarding to interaction between *Bacillus subtilis* and doses of gamma irradiation, *Bacillus subtilis* $10x10^{10}$ and $10x10^{12}$ detected with dose at 15 and 20 Gy were the most effective.

Effect of *Bacillus subtilis* and Certain Doses of Gamma Radiation on Peroxidase, Polyphenol Oxidase and Catalase Activity

As shown in Fig. 1 all treatments *Bacillus subtilis* $10x10^{10}$ and $10x10^{12}$ cfu/ml elevated peroxidase, catalase and polyphenol oxidase compared with control *i.e.,* The highest Peroxidase activity were observed with *B.subtilis* $10x10^{10}$ and $10x10^{12}$ cfu/ml (38 and 37 Unit/g fresh weight /5min) as compared with lowest enzyme activity in control treatment *i.e.,* untreated (22Unit/g fresh weight /5min)

Pertaining to poly phenol oxidase, the highest polyphanol oxidase activity was observed *B.subtilis* $10x10^{10}$ and $10x10^{12}$ cfu/ml (30 and 33 Unit/g fresh weight/5 min).

Pertaining to catalase activity, the highest catalase activity was observed *B.subtilis* $10x10^{10}$ (4 Unit/g fresh weight/3min) as compared with lowest enzyme activity in control treatment untreated (3Unit /g fresh weight/3 min). Generally *Bacillus subtilis* significantly increase antioxidant enzymes compared with control (untreated) are in agreement with **Yang *et al.* (2009)** who found that antioxidant enzymes catalase and peroxidase improved significantly 128% and 81.5% over control after inoculated eggplants with *Bacillus subtilis*.

Regarding to interaction between *Bacillus subtilis* and doses of gamma irradiation, *Bacillus subtilis* $10x10^{10}$ and $10x10^{12}$ detected with dose at 15 and 20 Gy were the most effective. Each figure represents the mean of three replicates.

Effect of *Bacillus subtilis* and Certain Doses of Gamma Radiation on Total Phenols

Data in Fig. 2 showed that all treatments *Bacillus subtilis* $10x10^{10}$cfu/ml.

Each figure represents the mean of three replicates and $10x10^{12}$ cfu/ml significantly elevated the total phenols 13and 14 mg/g fresh weight as compared with control *i.e.,* untreated 7 mg/g fresh weight. These results are in agreement with **Mahdy *et al.* (2006)** found that *Bacillus* filtrate increase total phenol (48.71% over control) against cucumber powdery mildew.

Regarding to interaction between gamma irradiation and *Bacillus subtilis*, the highest total phenols induced by *Bacillus subtilis* $10x10^{12}$at 20Gy.

Table (3-a): Effect of *Bacillus subtilis* and certain doses of gamma radiation on plant length (cm).

Plant Length (cm)						
***Bacillus subtilis* (cfu/ml)**	**0**		**10x10^{10}**		**10x10^{12}**	
Radiation (Gy)	**Seas.1**	**Seas.2**	**Seas.1**	**Seas.2**	**Seas.1**	**Seas.2**
0	28.3h	30.3j	30.3g	31.3j	31.7ef	32.3k
10	30.7fg	33gh	32.3de	33.3fgh	33.3cd	34fg
15	32.7de	35.7e	37b	40c	37.7ab	41.7b
20	34c	37.7d	38.3a	42.7ab	38.7a	43.3a
25	29.7g	30.3j	31.7ef	33.3fgh	33.3cd	34.3f

Means in the same column followed by different letters indicate significant difference (LSD, P>0.05).

Table (3-b): Effect of *Bacillus subtilis* and certain doses of gamma radiation on root length (cm).

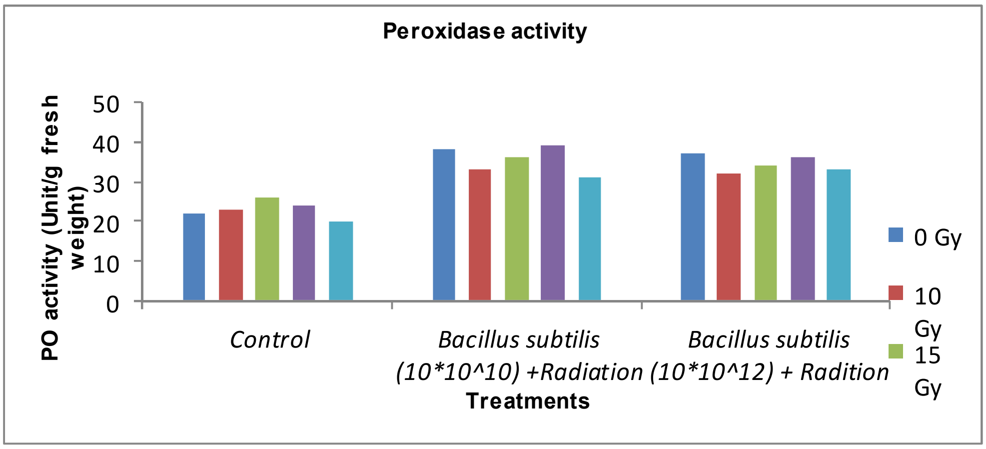
Root length (cm)						
***Bacillus subtilis* (cfu/ml)**	**0**		**10x10^{10}**		**10x10^{12}**	
Radiation (Gy)	**Seas.1**	**Seas.2**	**Seas.1**	**Seas.2**	**Seas.1**	**Seas.2**
0	5g	5.2h	5.3fg	5.5gh	6cde	6.2de
10	5.5efg	5.7fg	6cde	6.3cde	6.5cd	6.5cd
15	6cde	6.3cde	7.2b	7.2b	7.7ab	8.2a
20	6.3cd	6.7c	7.5ab	7.3b	7.8a	8a
25	5.5efg	5.7fg	6cde	6.2de	5.8def	6ef

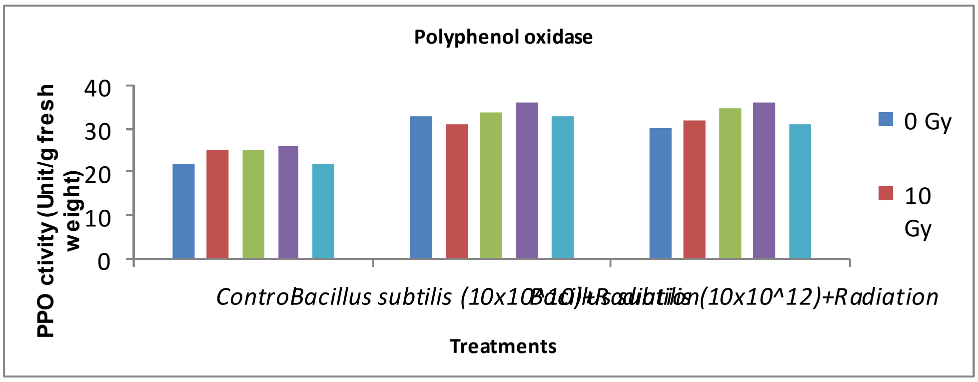
Means in the same column followed by different letters indicate significant difference (LSD, P>0.05).

Table (3-c): Effect of *Bacillus subtilis* and certain doses of gamma radiation on plant dray weight (g).

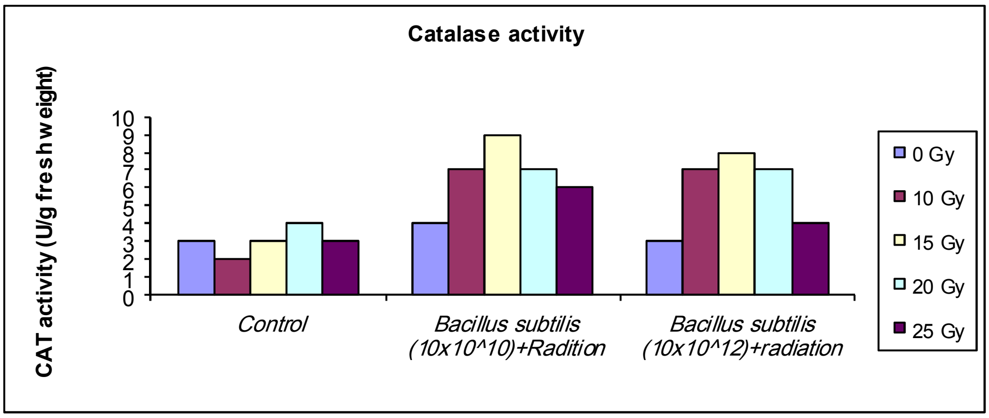
Plant dry weight (g)						
***Bacillus subtilis* (cfu/ml)**	**0**		**10x10^{10}**		**10x10^{12}**	
Radiation (Gy)	**Seas.1**	**Seas.2**	**Seas.1**	**Seas.2**	**Seas.1**	**Seas.2**
0	2.7k	2.9i	3.5fg	3.6ef	3.6f	3.7e
10	3i	3.3g	4e	4.1d	4.2d	4.2d
15	3.h2	3.5f	4.5b	4.7b	4.6a	4.8ab
20	3.5g	3.7e	4.7a	4.8a	4.7a	4.9a
25	2.8j	3.1h	4.1de	4.2d	4.3c	4.4c

Means in the same column followed by different letters indicate significant difference (LSD, P>0.05).





(B)



(C)

Fig. (1): Effect of *Bacillus subtilis* and certain doses of gamma irradiation on peroxidase activity (A), Polyphenol oxidase (B) and catalase activity (c).

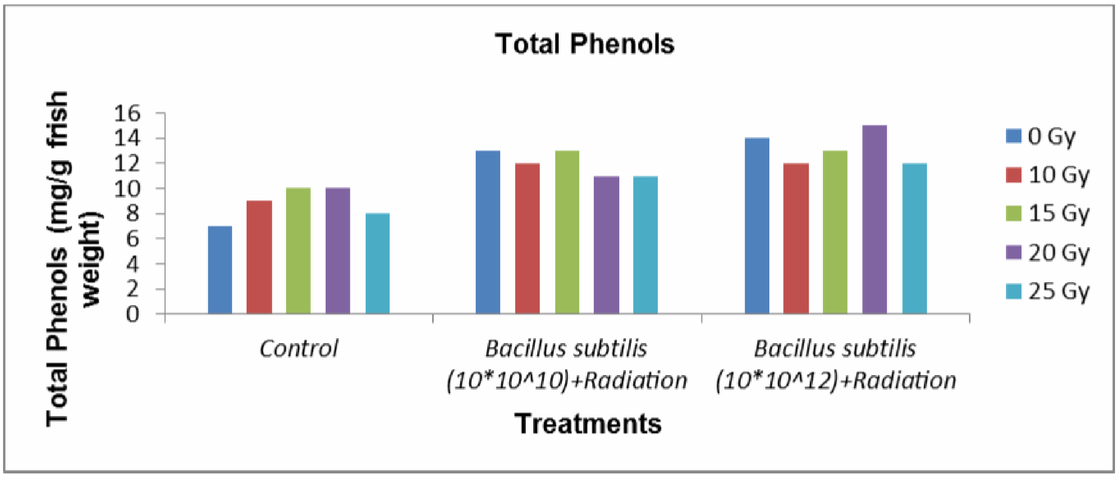


Fig. (2): Effect of *Bacillus subtilis* and certain doses of gamma radiation on total phenols

REFERENCES

Abd El-Moneim, M.L. (2004). Integrated system to protect cucumber plants in greenhouses against diseases and pests underorganic farming conditions. Egypt. J. Agric. Res., 82 (2): 1-9.

Abdel-Sayed, M.H.F. (2000). Studies on powdery mildew disease of cucurbits under protected cultivation. M.Sc. Thesis, Dept. Plant Path., Fac. Agric., Cairo Univ., Egypt.

Ahmed, A.S.Y. (1995). Studies on the powdery mildew disease of cucurbits. M.Sc Thesis, Fac. of Agric., Al-Azhar Univ. and plant extracts. J. Agric. Sci., 2: 220-230.

Angelini, R.; Manes, F. and Federico, R. (1990). Spatial and functional correlation between *iamine-oxidase* and *peroxidase activities and their dependence upon de-*etiolation and wounding in chick-pea stems. Planta., 182: 89- 96.

Borzouei, A.; Kafi, M.; Khazaei, H.; Naseriyam, B. and Majedabadi, A. (2010). Effect of Gamma radiation on germination and physiological wheet (*Triticum aestivum* L.) seedling, Pak. J. Bot., 42(4): 2281- 2290.

Compants, S.; Duffy, B.; Nowak, J.; Clement, C. and Barka, E.A. (2005). diseases: Principles, mechanisms of action, and future prospects, Appl. and Environ. Microbiol., 71: 4951-4959.

Elad, Y. (2000). Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. Crop Prot., 19:709-714.

Esterbauer, H.; Schwarzl, E. and Hayn, M. (1977). A rapid assay for Factors affecting acquired resistance in *Erysiphe cichoracearum* the causal organism of cucumber powdery mildew. Agric. Res. Rev., 68 (3): 521-528.

Garg, O.K.; Misra, B.C. and Singh, B.P. (1972). Effect of pre- sowing exposure of seeds to Gamma radiation on the drought resistance behavior of barley plants. Plant and Soil, 36: 39-45.

Hanam, J.J., Holley, W.D. and Goldsberry, K.L. (1978). Greenhouse management, Springer-Verlag, Berlin. Heidelberg and New York, 530.

Kim, J.; Beak, M and Lee, Y. (2001). Effects of low dose gamma radiation on the dormancy, breaking and physiological activity of (Dejima) seed potato. Chungnam National Univ., Taejon (Republic of Koria) 24-25 May, 8 ref. 4

figs, 1 tab. Avaialble from kns, Taejon (Koria).

Mahdy, A.; Abd El-Mageed, M.; Hafez, M. and Ahmed, G. (2006). Using alternatives to control cucumber powdery mildew under green- and commercial protected-house conditions. Fayoum J. Agric. Res. and Dev., 20 (2): 121-138.

Meliki, M. and Marouani, A. (2010). Effect of Gamma rays irradiation on seed germination and growth of hard wheat. Environ. Chem. Lett., 8 (4): 307-310.

Mosa, A.A. (1997). Effect of foliar application of phosphate on cucumber powdery mildew. Annals. Agric. Sci. Ain Shams Univ. Cairo, 42:241-255.

Mukhin, V.; Gerpuski, D. and Selman, V. (1979). Effect of radiation main spear fresh and dry weight of broccoli and invrease of productivity of vegetables grown in either type. Rusting J. Pochvoret, 25: (3) 44- 47.

Nair, K.R.; Sivan, S. and Ellingboe, A.H. (1962). A method of controlled inoculations with conidiospores of *Erysiphe graminis* var. *tritici*. Phytopathol., 52:714.

Reuveni, R.; Dor, G.; Raviv, M.; Reuveni, M. and Tuzun, S. (2000). Systemic resistance against Sphaerotheca fuliginea in cucumber plants exposed to phosphate in hydroponics system and its control by foliar spray of mono-potassium phosphate. Crop Prot., 19: 355-361

Singleton, V.L. and Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Ame. J. Enol. Vitic., 16 (3): 144-158.

Snedecor, G.W. and Cochran, W. (1982). Statistical Methods 7th Ed. Aiwa State Univ. Press. Ame. Aiwa. USA.

Tkemaladaze, G. and Esitadeze, K.G. (1975). Enzymology Practical Tbilis in Georgian, 298.

Veeresh, L.; Shivashankar, H.; Shai, L. and Hittalmani, S. (1995). Effect of seed irradiation on some plant characteristics of winged bean. J. Agric. Sci., 29:1-4.

Yang, Z.; Liu, X.; Liu, Z.; Pang, J.; Qiu, J. and Yang, W. (2009). Effect of *Bacillus Subtilis* SY1 on antifungal activity and plant growth. Int. J. Agric. and Biol. Eng., 2 (4): 55-61.

Zatarin, M.; Cardoso, A. and FU, I. (2005). Horticultura Brasileira Rtado, E.L. Effitosde tipos de letie sorbe oidio en abobora plantadas no campo., 23 (2): 198-201.

الملخص العربى

استخدام بدائل لمقاومة مرض البياض الدقيقى فى بذور الخيار المعاملة بالإشعاع

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استحثاث المقاومة ضد مرض البياض الدقيقى الذى يسببه فطر *Sphaerotheca fuliginea* فى بذور الخيار من صنف بيتا الفا المشععة بأشعة جاما بجرعات منخفضة لتحفيز النمو حيث تم استخدام بكتيريا الباسللس سابتلس كمعامل حيوى للتحكم فى المرض وإستخدم الماء ككنترول وتم قياس شدة المرض وقياس نشاط بعض الإنزيمات مثل إنزيم البيروكسيديز والكتاليز والبولى فينول اوكسيديزوالمحتوى الكلى للفيولات وأيضا تم قياس بعض النموات الخضرية مثل طول النبات وطول الجذر والوزن الجاف للنبات, حيث أدت المعاملة بالباسيللس إلى زيادة نشاط الإنزيمات والمحتوى الكلى للفينول مقارنة بالكنترول وتحقق مع الجرعتين ١٥و٢٠ جراى أفضل تحفيز للنمو وكانتا أفضل من الجرعتين ١٠و٢٥ جراى.

الكلمات الأسترشادية: البياض الدقيقى، بذور الخيار، المعاملة بالإشعاع.

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