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COMBINED EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA AND GAMMA RADIATION ON GROWTH AND ESSENTIAL OIL PRODUCTIVITY OF FENNEL (*FOENICULUM VULGARE* L.)

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ABSTRACT

Seeds of fennel (*Foeniculum vulgare*, Miller) were irradiated with gamma rays at doses of 0, 50, 100 and 150 Gray. Irradiated and non-irradiated fennel seeds were soaked in bacterial suspension of *B. subtilis*, *P. fluorescens*, *Sinorhizobium* and *Bradyrhizobium* for 15 min. Treated and non-treated seeds were cultivated in sandy loam soil at two successive seasons of 2008 and 2009. At full blooming stage, fresh and dry weights of plant shoots were recorded. However, at full maturity, fruits were harvested and seed yield was recorded. Essential oil percent was determined in fennel seeds and oil yield was calculated. GLC was carried out for essential oils extracted from non-irradiated and irradiated seeds at 100 Gray under the different bacterial inoculation. In addition, sugars and chlorophyll contents were determined in fennel plant shoots. Results showed that all the applied treatments had the capacity to stimulate plant growth as it increased fresh and dry weights of the grown plants and intern increased fruits and seeds yield. Essential oil production was increased due to the enhancement of seeds yield. However, no effect was detected in essential oil constituents in response to the applied treatments. Irradiation dose of 100 Gray and bradyrhizobium inoculation were found to be the superior treatments to improve plant growth and maximizing oil production during the two studied seasons.

Keywords: fennel, *Foeniculum vulgare*, Miller, gamma rays, *B. subtilis*, *P. fluorescens*, *Sinorhizobium* and *Bradyrhizobium,* Essential oil, yield,

INTRODUCTION

Fennel (*Foeniculum vulgare*, Miller) is one of the most important medicinal and aromatic plants due to its medical and pharmaceutical applications. Fennel volatile oil is recommended to be used for increasing milk secretion, facilitate birth, flatulent dyspepsia, anorexia, carminative distending pain in the epigastrium with anorexia, diuretic, appetizer in addition to other economic and industrial applications.

Plant growth promoting rizobacteria (PGPR) comprise a diverse group of rizosphere–coloning bacteria and diazotrophic microorganisms which, when grown in association with a plant, stimulate growth of the host. PGPR can affect plant growth and development indirectly or directly (Glick, 1995 and Vessey, 2003). In concern with indirect promotion, the bacteria decrease or eliminate certain deleterious effects of a pathogenic microorganism through various mechanisms, including induction of host resistance to the pathogen as illustrated by Van Loon and Glick (2004) and Van Loon (2007).

Concerning direct promotion, the bacteria may provide the host plant with synthesized compounds; facilitate uptake of nutrients; fix atmospheric nitrogen; solubilize minerals such as phosphorus; produce siderophore which solubilize iron, synthesize phytohormones, including auxins, cytokinins, and gibberellins, which enhance various stages of plant growth; or synthesize enzymes that modulate plant growth and development (Lucy *et al.,* 2004; Gray and Smith, 2005).

Bradyrhizobium and *Sinorhizobium* which belong to family rhizobiaceae, are known for their ability to fix atmospheric nitrogen while living symbiotically on and nodulating the roots of leguminous plants. However, members of this family also display non-specific associative interactions with roots of other plants, without forming nodules (Van Loon, 2007). These rizobial strains are presumed to produce plant growth regulators and are classified as PGPR as mentioned by Vessey (2003).

Methods for enhanced growth of economically important plants are constantly evolving. Widespread use of chemical nitrogen fertilizer has increased crops productivity, but has also caused deleterious effects on ecosystems, i.e., nitrate (NO_3) pollution of

ground and surface waters, soil acidification, and production of the greenhouse gas nitrous oxide (N_2O) through denitrification as illustrated by (Biswas *et al.,* 2000). In order to reduce such negative environment effects, there has been a recent call for organic agricultural practices "Organic agriculture" is a production system, which avoids or minimizes the use of synthetic fertilizers, pesticides, and growth regulators.

On the other side, the stimulating effects of ionizing radiation on plant growth following pre-planting irradiation has been documented via numerous reports. Faster vegetative growth, early flowering and increasing crops productivity are the most common reported results. Recently, ionizing radiation is used for stimulating growth and increasing active ingredients of medicinal and aromatic plants as mentioned by Hassanein (2003) and Deaf (2007).

In the search for new strategies of plant production with high yield without undesirable effects, it is important to investigate unconventional alternatives such as inoculation with PGPR and exposing to the recommended low stimulating doses of gamma radiation. The purpose of the present study is to document the response of fennel plant to gamma radiation and inoculation with various PGPR strains.

MATERIALS AND METHODS

Plant Material:

Fresh harvested seeds of fennel (*Foeniculum vulgare*) were obtained from Fac. of Pharmacy, Cairo Univ.

Irradiation Treatments:

Fennel seeds were exposed to gamma irradiation doses of 0, 50, 100 and 150 Gray. Irradiation was carried out in Egypt's Mega Gamma-1, type J 6600 cobalt-60 cell installed at National Center for radiation and Technology, Nasr city, Cairo, Egypt.

Bacterial strains, culture conditions and inoculation:

Four strains which are reported as plant growth promoting bacteria (Lucy *et al.,* 2004, and Van Loon 2007) were assessed for PGPR effects; *Pseudomonas fluorescens* WCS 417r, *Bacillus subtillis* 09, *Sinorizobium meliloti* Rm 1021, and *Bradyrhizobium sp.* USDA 4438.

These bacteria were grown on TY (5g/L. tryptone, 3g/L. yeast extract, 1g/L. $CaCl_2$). TSA (15g/L. tryptone, 5g/L. soy peptone, 5g/L. NaCl). RDM (5g/L. sucrose, 0.5g/L. yeast extract,0.6g/L. KNO_3, 0.1g/L $CaCL_2$, 0.25g/L. Mg SO_4, 1g/L. K_2HPO_4, 1g/L. KH_2PO_4, 0.01g/L. Fe Cl_3) as prepared by Alagawadi and Gaur (1988) and Bogino *et al.,* (2006), and TY media, respectively for routine use, and maintained in nutrient broth with 15% glycerol at -80^{o}C for long-term storage. For experiments, bacteria were grown agar plates; single colonies were transferred to 100 ml flasks containing respective culture media, and grown aerobically on a rotating shaker (150 rpm) for 48h at 28^{o}C.The bacterial suspension was diluted in sterile saline solution (0.9 % Na Cl) to final concentration of 10 CFU ml-^{1}. Irradiated and non-irradiated fennel seeds were soaked in the different bacterial suspension for 15 min and then were cultivated in the experimental field.

Field experiment:

Treated and non-treated seeds were sown in sandy loam soil at the experimental field of AEA, Inshas during two successive seasons of 2008 and 2009. Treatments were arranged in a randomized complete block design with three replicates. Plot area was 10.5 m² (3×3.5 m) and each plot contained 30 plants. All the agricultural practices were carried out to the normal system for fennel cultivation. Fresh and dry weights of plant shoots were recorded during the full blooming stage. At full maturity, fruits were harvested and seed yields for plots was weighed.

Essential oil extraction:

Essential oil content of the air-dried seeds of fennel was extracted by water distillation for 5-6 h according to the method of British Pharmacopia (1963). After distillation, the oil was dried over anhydrous sodium sulphate and kept in a deep freezer at 2^{o}C until analysis.

GLC of Fennel Essential Oil:

Hewlett-Packard gas chromatography equipped with FID and fused silica capillary column DB-5 (30m × 0.320 mm × 0.5 µm film thickness) was used for separating essential oil components of fennel seeds. The separated components were identified by comparing their retention time with that of reference material. Area normalization

method was used for the qualitative analysis of the identified components.

Determination of chlorophyll content:

Chlorophyll content of fennel leaves was extracted and determined according to Wintermans and Mots (1965).

Determination of total sugars content:

Total sugars content was extracted from fennel leaves using 70% ethanol according to Kawamura *et al.,* (1966). Total sugars was colorimetrically determined according to Dubois *et al.,* (1956).

Statistical analysis:

Analysis of variance for the final data was carried out according to Snedecor and Cohran (1982).

RESULTS AND DISCUSSION

1-Shoots weight:

Fresh and dry weights of fennel plants as affected by pre-planting gamma irradiation and rhizobacterial inoculation are presented in table (1). Data of the first season (2008), clearly reveal that both the applied treatments significantly stimulated fennel growth as it increased the fresh and dry weights of plant shoots. Regarding the influence of the imposed gamma irradiation treatments, it could be easily deduced that irradiation dose of 100 Gy was the most superior dose to enhance fresh and dry weights of fennel shoots followed by irradiation doses of 150 and 50 Gy. The stimulation effect of the low doses of gamma radiation on plant growth is established and has been documented by several investigators as mentioned by Hassanein (2003), Deaf (2007), and Taha (2009). On the other side, inoculation of the irradiated and non-irradiated fennel seeds with the different strains of the studied rhizobacteria showed further enhancement in fresh and dry weight of the produced plant shoots. Maximum stimulation was exerted by seed inoculation with *Brandyrhizobium* followed by *P. fluorescens*, *Sinorhizobium* and *B. subtilis*, respectively. That holds true for both fresh and dry weights of fennel shoots. The beneficial effect of the plant growth promoting rhizobacteria has been explained by Banchio *et al.,* (2008).

In this consideration, rhizobacteria inoculation promote plant growth as it can eliminate deterious effects of some pathogenic effect

(Van Loon, 2007); facilitate uptake of nutrients; fix atmospheric nitrogen; synthesize of phytohormones and enzymes that modulate plant growth and development (Gray and Smith, 2005). Regarding the interaction effect of both gamma irradiation doses and rhizobacterial inoculation, it could be noticed that the maximum fresh or dry shoots weight was resulted from irradiation dose of 100 Gy when interacted with bradyrhizobium inoculation. Data of second season (2009) showed similar trends and results as those shown for 2008 which confirm the influences of the studied treatments on fennel plant growth and development. Only, the values of the fresh and dry weights fennel shoots were slightly higher in the second season rather than the first one. Such variation is expected and should be attributed to various parameters including the environmental and atmospheric conditions in both season.

Table (1): Effect of seed inoculation with plant growth promoting rhizobacteria and gamma radiation on fresh and dry weight of fennel shoots.

Rhizobacteria strains	Shoots fresh Weight					Shoots dry Weight				
	Irradiation doses in Gy									
	0	50	100	150	Mean	0	50	100	150	Mean
First Season										
Control	298.5	314.7	332.8	326.2	381.1	74.8	78.5	83.9	81.8	79.8
B. subtilis	314.4	325.3	345.6	331.9	329.3	81.7	84.3	86.1	83.2	83.8
P. fluorescens	343.6	352.8	377.9	361.5	358.9	85.8	88.6	94.8	91.8	90.3
Sinorhizobium	321.7	335.3	345.5	339.6	335.5	83.2	83.9	86.8	84.8	84.7
Bradyrhizobium	354.6	361.5	383.8	376.4	369.1	85.4	90.2	95.8	94.7	91.5
Mean	326.6	337.9	357.1	347.1		82.2	85.1	89.5	87.3	
Second Season										
Control	314.8	323.5	347.4	355.8	330.4	78.3	81.3	86.8	83.9	82.6
B. subtilis	327.5	341.4	368.6	356.4	348.5	85.4	86.8	92.2	89.4	88.6
P. fluorescens	354.4	363.5	381.4	372.6	367.9	84.9	91.2	95.7	92.8	91.2
Sinorhizobium	336.2	341.3	362.1	355.4	348.8	83.2	85.7	91.1	88.9	87.2
Bradyrhizobium	361.5	375.4	396.2	383.4	379.1	90.8	94.8	101.3	96.8	95.9
Mean	338.9	349.0	371.1	360.7		84.5	87.9	93.4	90.4	

L.S.D. (5%)

	Fresh weight		Dry weight	
	First season	Second season	First season	Second season
Bacteria	8.2	9.4	2.1	2.4
Radiation	9.4	11.2	2.4	2.7
Interaction	16.8	11.2	4.6	5.9

2-Seeds yield:

Seeds yield of fennel plant (expressed as kg/plot or kg/fed) is demonstrated in Table (2). For both the first and second seasons (2008 & 2009), data clearly reveal that seeds yield was significantly increased due to the effect of both the applied treatments. In this respect, all the applied irradiation doses had the capacity to enhance seed yield of fennel plant. In this consideration, irradiation dose of 100 Gy exhibited the maximum fennel yield followed by dose of 150 and 50 Gy. That holds true for both the first and second seasons. Previous studies exhibited the stimulation effect of gamma radiation to increase the seeds yield of other medicinal and aromatic plants as showed recently by Deaf (2007) and Taha (2009).

On the other side, treating fennel seeds with the different strains of *Rhizobacterial inoculum* before sowing showed promising and significant increase in the resulted yield and that holds true for the two consequent seasons. Maximum fennel seeds yield was exerted by *Bradyrhizobium inoculum* followed by *P. fluorescens*, *Sinorhizobium* and *B. subtilis*, respectively. Magnitude of increase due to bradyrhizobium inoculation was 31.6 and 28.5% over the control for the first and second seasons, respectively. The promising effect of *Rhizobacterial inoculum* to enhance and increase yield of further crops was confirmed by Singh and Kapoor (1999), Prasad *et al.,* (2005) and El-Hadidy *et al.,* (2006).

Table (2): Effect of seed inoculation with plant growth promoting rhizobacteria and exposing to gamma radiation on seeds yield of fennel plant.

Rhizobacteria strains	Kg / plot.					Kg / fed.				
	Irradiation doses in Gy									
	0	50	100	150	Mean	0	50	100	150	Mean
First Season										
Control	1.053	1.131	1.286	1.214	1.171	402.3	432.1	491.3	463.8	447.4
B. subtilis	1.135	1.286	1.452	1.322	1.299	433.7	491.3	554.8	505.1	496.2
P. fluorescens	1.316	1.524	1.761	1.642	1.561	502.8	582.3	672.8	627.3	596.3
Sinorhizobium	1.255	1.391	1.569	1.183	1.425	479.5	531.4	599.5	566.6	544.3
Bradyrhizobium	1.398	1.621	1.938	1.801	1.690	534.1	619.3	740.4	688.1	645.5
Mean	1.231	1.391	1.601	1.492		470.5	531.3	611.8	570.2	
Second Season										
Control	1.138	1.289	1.465	1.380	1.318	434.8	492.5	559.7	527.2	503.6
B. subtilis	1.261	1.390	1.568	1.481	1.425	481.8	531.1	599.1	565.8	544.5
P. fluorescens	1.468	1.609	1.829	1.752	1.665	560.9	614.7	698.8	669.4	636.0
Sinorhizobium	1.356	1.514	1.761	1.702	1.583	518.1	578.4	672.8	650.3	604.9
Bradyrhizobium	1.533	1.781	2.083	1.976	1.843	585.7	680.5	795.8	754.9	704.2
Mean	1.351	1.517	1.741	1.658		516.3	479.4	665.2	633.1	

L.S.D. (5%)	Kg / plot.		Kg / fed.	
	First season	Second season	First season	Second season
Bacteria	0.286	0.293	15.2	17.8
Radiation	0.314	0.381	18.9	21.6
Interaction	0.503	0.588	31.8	35.8

3-Essential oil content:

Data of essential oil content expressed as cc/100 g. seeds or cc/plant are presented in Table (3). Essential oil percent (cc/100 g.) showed no significant effect due to the imposed treatments. In other words, essential oil percent showed fixed phenomenon towards both gamma radiation and rhizobacterial inoculation. Only, slight fluctuations could be noticed. That holds true for both the consequent seasons. Similar conclusions were mentioned by Deaf (2007) and Taha (2009) on other aromatic seed crops. Regarding the oil content (cc/plant), another impression could be noticed. Both imposed treatments statistically increased essential oil content (cc/plant). In this regard, all the applied irradiation doses induced significant increase in essential oil content and dose of 100 Gy was the most superior one. Similarly, inoculated fennel seeds with the studied rhizobacterial strains before sown exhibited an increase in essential oil content/plant.

In this consideration, bradyrhizobium inoculation induced the maximum oil content /plant for both the consequent seasons. Increasing essential oil content due to rhizobacterial inoculation was mentioned by El-Hadidy *et al.,* (2006) and Banchio *et al.,* (2008).

4-Essential oil yield:

Data presented in Table (4) demonstrate the impact of either gamma irradiation or inoculation with rhizobacteria on essential oil yield (expressed as cc/plot and liter/fed.) of fennel plant.

All the applied irradiation doses statistically increased essential oil yield of fennel and maximum oil yield was exerted by dose of 100 Gy followed by those of 150 and 50 Gy, respectively. Volume of increase in essential oil yield (L/fed.) due to irradiation dose of 100 Gy was 34.9 and 32.15 %, for the first and second seasons, respectively.

Enhancement of essential oil production in aromatic plants due to gamma radiation application was confirmed recently by Deaf (2007) and Taha (2009).

On the other side, rhizobacterial inoculation of irradiated and non-irradiated fennel seeds before sown induced further increase in essential oil yield. Again, bradyrhizobium inoculation was the most superior strain to enhance essential oil production of fennel plant followed by *P. fluorescens, sinorhizobium* and *B. subtilis* inoculation. Maximum oil yield was resulted from 100 Gy gamma irradiation and bradyrhizobium inoculation as it was 20.93 and 23.23 L. /fed. For the first and second season, respectively. The present results are in harmony with those of Novak *et al.,* (2006), El-Hadidy *et al.,* (2006) and Banchio *et al.,* (2008).

Table (3): Effect of seed inoculation with plant growth promoting rhizobacteria and exposing to gamma radiation on essential oil of fennel seeds.

Rhizobacteria strains	Essential oil percent (cc/100 g. seeds)					Essential oil percent (cc/plant)				
	Irradiation doses in Gy									
	0	50	100	150	Mean	0	50	100	150	Mean
First Season										
Control	2.58	2.61	2.72	2.63	2.64	0.85	0.92	1.06	0.99	0.96
B. subtilis	2.61	2.68	2.79	2.66	2.69	0.95	1.08	1.21	1.10	1.09
P. fluorescens	2.71	2.79	2.81	2.74	2.76	1.13	1.33	1.55	1.41	1.36
Sinorhizobium	2.75	2.71	2.76	2.61	2.71	1.08	1.17	1.35	1.21	1.20
Bradyrhizobium	2.78	2.81	2.83	2.81	2.81	1.21	1.42	1.71	1.58	1.48
Mean	2.69	2.72	2.78	2.69		1.05	1.18	1.38	1.26	
Second Season										
Control	2.62	2.68	2.75	2.66	2.68	0.93	1.08	1.26	1.15	1.11
B. subtilis	2.64	2.71	2.83	2.71	2.72	1.04	1.18	1.38	1.25	1.21
P. fluorescens	2.75	2.82	2.85	2.79	2.80	1.26	1.42	1.63	1.53	1.46
Sinorhizobium	2.79	2.81	2.88	2.69	2.79	1.18	1.33	1.58	1.43	1.38
Bradyrhizobium	2.81	2.83	2.92	2.71	2.82	1.35	1.63	1.90	1.67	1.64
Mean	2.72	2.77	2.85	2.71		1.15	1.33	1.55	1.41	

L.S.D. (5%)

	Essential oil percent (cc/100 g. seeds)		Essential oil percent (cc/plant)	
	First season	Second season	First season	Second season
Bacteria	N.S	N.S	0.14	0.18
Radiation	N.S	N.S	0.26	0.36
Interaction	N.S	N.S	0.45	0.52

Table (4): Effect of seed inoculation with plant growth promoting rhizobacteria and exposing to gamma radiation on essential oil yield of fennel seeds.

Rhizobacteria strains	cc / plot					Liter / fed.				
	Irradiation doses in Gy									
	0	50	100	150	Mean	0	50	100	150	Mean
First Season										
Control	27.2	29.6	34.9	31.9	30.9	10.39	11.31	13.33	12.19	11.81
B. subtilis	29.6	34.5	40.5	35.2	34.9	11.31	13.18	15.47	13.45	13.35
P. fluorescens	35.7	42.5	49.5	40.6	42.1	13.64	16.24	18.91	15.51	16.08
Sinorhizobium	34.5	37.7	43.3	38.7	38.6	13.18	14.40	16.54	14.78	14.73
Bradyrhizobium	38.9	45.6	54.8	50.6	47.5	14.86	17.42	20.93	19.33	18.14
Mean	33.2	38.0	44.6	39.4		12.67	14.51	17.03	15.05	
Second Season										
Control	29.8	34.5	40.3	36.7	35.3	11.38	13.18	15.39	14.02	13.49
B. subtilis	33.3	37.5	44.4	40.1	38.9	12.72	14.40	16.96	15.32	14.85
P. fluorescens	40.4	45.4	52.1	48.9	46.7	15.43	17.34	19.90	18.68	17.84
Sinorhizobium	37.9	42.5	50.7	45.8	44.2	14.47	16.24	19.37	17.50	16.90
Bradyrhizobium	43.1	50.4	60.8	53.5	52.0	16.46	19.25	23.23	20.44	19.85
Mean	36.9	42.1	49.7	45.0		14.09	16.08	18.97	17.19	

L.S.D. (5%)

	cc / plot		Liter / fed.	
	First season	Second season	First season	Second season
Bacteria	2.67	2.86	0.92	0.97
Radiation	3.14	3.81	1.21	1.32
Interaction	5.89	6.17	2.76	2.91

5-Essential oil constituents:

Data presented in Tables (5) & (6) demonstrate the impact of the most stimulant dose of 100 Gy gamma radiation and plant growth promoting rhizobacteria on essential oil constituents of fennel seeds. Generally, data revealed that 11 components were resulted which 9 of them were identified and 2 were unknowns. Trans-anethole, limonene and methyl chavicol were the most predominant components. Whereas, cis-anethole, p-anisic acid, fenchone and α-pinene were found in relatively moderate or minor ratios. On the other hand, the rest of identified constituents were found in concentrations less than 1%. Similar results were mentioned by Pietro *et al.,* (2004), Lado *et al.,* (2005) and Misharina and Polshkov (2005) as they identified the same constituents in more or less ratios.

Regarding the effect of the studied treatments on essential oil constituents, it could be easily deduced that limited and minor variation could be shown. In other words, simple fluctuations could be observed and that holds true for both the consequent seasons. For example, limonene showed minor reduction under *B. subtilis* and sino-rhizobium inoculation. However, Methyl chavicol and Trans-anethole were slightly increased under the same inoculations. That holds true for non-irradiated and 100 Gy irradiated seeds. Similarly, fluctuations could be noticed for the other identified components.

Deaf (2009) and Taha (2009) came to the same conclusion regarding the effect of pre-sowing gamma radiation on essential oil constituents of other aromatic plants. Also, Gewaily *et al.,* (2006) and Banchio *et al.,* (2008) recorded that bacterial inoculation or biofertilization had no considerable effect on essential oil constituents of further aromatic plants.

Table (5): Gas –liquid chromatography of fennel seeds oil as affected by pre-planting seed inoculation with plant growth promoting rhizobacteria and gamma radiation (First season).

Peak No.	Component	RRT*	Non-irradiated seeds					Seeds exposed to 100 Gy				
			Control	*B. subtilis*	*P. fluorescens*	*Sino-rhizobium*	*Brady-rizobium*	Control	*B. subtilis*	*P. fluorescens*	*Sino-rhizobium*	*Brady-rizobium*
1	α - pinene	0.26	1.84	1.73	1.78	1.69	1.70	1.77	1.73	1.69	1.62	1.71
2	Fenchone	0.29	0.26	0.31	0.33	0.29	0.25	0.31	0.30	0.38	0.33	0.36
3	Limonene	0.34	12.72	12.61	11.89	11.31	11.98	11.84	10.62	11.21	11.08	11.75
4	Methyl chavicol	0.58	5.31	4.92	4.77	4.33	4.81	5.81	5.73	5.69	5.71	5.88
5	Unknown	0.74	3.66	3.56	3.71	3.62	3.54	4.21	3.92	3.89	3.79	4.11
6	Unknown	0.89	0.11	0.18	0.16	0.17	0.21	0.16	0.18	0.14	0.13	0.15
7	Trans anethole	1.00	75.10	75.24	75.9	77.2	76.12	74.4	76.09	75.46	75.90	74.67
8	Carvone	1.11	0.21	0.28	0.29	0.24	0.19	0.26	0.22	0.27	0.29	0.24
9	Cis-anethole	1.91	0.11	0.16	0.18	0.14	0.17	0.17	0.21	0.24	0.14	0.19
10	Anise Aldhyde	1.30	0.83	0.77	0.73	0.76	0.80	0.76	0.71	0.76	0.79	0.69
11	P. Anisic acid	1.49	0.21	0.24	0.26	0.25	0.23	0.31	0.29	0.27	0.22	0.25

*RRT = Relative Retention Time and Trans anethole was given value of 1.00

Table (6): Gas –liquid chromatography of fennel seeds oil as affected by pre-planting seed inoculation with plant growth promoting rhizobacteria and gamma radiation (Second season).

Peak No.	Component	RRT*	Non-irradiated seeds					Seeds exposed to 100 Gy				
			Control	*B. subtilis*	*P. fluorescens*	*Sino-rhizobium*	*Brady-rizobium*	Control	*B. subtilis*	*P. fluorescens*	*Sino-rhizobium*	*Brady-rizobium*
1	α - pinene	0.28	1.91	1.76	1.81	1.77	1.84	1.84	1.62	1.74	1.69	1.79
2	Fenchone	0.31	0.29	0.26	0.28	0.22	0.27	0.26	0.31	0.24	0.28	0.22
3	Limonene	0.38	13.56	12.54	13.11	12.84	11.16	12.65	11.47	11.69	11.81	12.02
4	Methyl chavicol	0.61	4.89	5.12	4.81	5.08	4.96	5.22	4.96	4.88	4.79	4.81
5	Unknown	0.78	3.16	3.22	3.59	3.22	3.34	3.56	3.41	3.48	3.38	3.49
6	Unknown	0.91	0.14	0.17	0.22	0.16	0.17	0.16	0.11	0.15	0.17	0.10
7	Trans anethole	1.00	74.71	75.77	75.01	75.44	76.99	75.08	76.89	76.44	76.62	76.42
8	Carvone	1.14	0.18	0.09	0.11	0.14	0.13	0.16	0.12	0.21	0.19	0.11
9	Cis-anethole	1.21	0.09	0.12	0.07	0.12	0.08	0.08	0.11	0.10	0.13	0.09
10	Anise Aldhyde	1.31	0.89	0.74	0.81	0.77	0.84	0.78	0.76	0.81	0.75	0.73
11	P. Anisic acid	1.59	0.18	0.21	0.19	0.24	0.22	0.21	0.24	0.26	0.19	0.22

*RRT = Relative Retention Time and Trans anethole was given value of 1.00

6-Total chlorophyll content:

Results of total chlorophyll content of fennel shoots as affected by rhizobacterial inoculation and pre-sowing gamma radiation are presented in Table (7). Data clearly demonstrate that either the imposed treatments have significant effect on total chlorophyll content of fennel shoots. In this consideration, all the rhizobacterial inoculation significantly increased the total chlorophyll content. That holds true for both the studied seasons. Maximum chlorophyll enhancement was exerted by *Brandyrhizobium* followed by *Sinorhizobium*, *B. subtilis* and *P. fluorescens*, respectively.

On the other side, all the applied gamma irradiation doses had the capacity to stimulate chlorophyll synthesis in fennel shoots. Again, irradiation dose of 100 Gy was the superior treatment to increase chlorophyll content. That holds true for two successive seasons. Maximum chlorophyll content was achieved by interaction of

bradyrhizobium inoculation and 100 Gy gamma irradiation. In this consideration, the stimulation effect of gamma radiation to enhance chlorophyll synthesis was recently recorded by Deaf (2007) and Taha (2009).

Table (7): Effect of seed inoculation with plant growth promoting rhizobacteria and exposing to gamma radiation on total chlorophyll and sugars content of fennel plant.

Rhizobacteria strains	Total chlorophyll content (mg/g.d.wt.)					Total sugars content (mg/g.d.wt.)				
	Irradiation doses in Gy									
	0	50	100	150	Mean	0	50	100	150	Mean
First Season										
Control	4.86	5.11	5.23	4.96	5.04	33.61	35.28	38.24	36.52	35.91
B. subtilis	5.08	5.28	5.52	5.11	5.25	36.14	40.16	45.78	42.83	41.23
P. fluorescens	4.96	5.17	5.38	4.98	5.12	32.52	33.18	35.81	33.16	33.67
Sinorhizobium	5.12	5.21	5.63	5.41	5.34	38.16	43.65	48.62	45.14	43.89
Bradyrhizobium	5.18	5.28	5.78	5.61	5.46	37.19	41.16	44.82	42.35	41.38
Mean	5.04	5.21	5.51	5.21		35.52	38.69	42.65	40.00	
Second Season										
Control	5.21	5.38	5.81	5.52	5.48	36.08	37.11	40.53	38.69	38.10
B. subtilis	5.34	5.52	5.96	5.71	5.63	39.14	43.81	46.11	43.15	43.05
P. fluorescens	5.28	5.41	5.76	5.63	5.52	36.91	38.18	42.53	40.16	39.45
Sinorhizobium	5.38	5.64	6.09	5.92	5.76	40.12	42.65	47.08	44.13	43.50
Bradyrhizobium	5.41	5.77	6.21	5.98	5.84	41.16	42.96	48.15	43.18	43.87
Mean	5.32	5.54	5.97	5.75		38.68	40.94	44.88	41.86	

L.S.D. (5%)

	Total chlorophyll content (mg/g.d.wt.)		Total sugars content (mg/g.d.wt.)	
	First season	Second season	First season	Second season
Bacteria	0.12	0.17	0.56	0.63
Radiation	0.26	0.36	0.67	0.71
Interaction	0.48	0.69	1.21	1.38

7-Total sugars content:

Total sugars content shown in Table (7) demonstrate that either the applied treatments had noticeable effect on total sugars content of fennel shoots. Statistically, the imposed treatments significantly stimulated sugars synthesis in fennel shoots. Thus, all the rhizobacterial inoculations increased sugars content in the studied plant and maximum enhancement was exerted by *Sinorhizobium* and Bradyrhizobium in the first and second seasons, respectively.

On the other side, the investigated gamma irradiation doses remarkably stimulated sugars synthesis in fennel shoots. Maximum stimulation was exerted by irradiation dose of 150 Gy followed by that of 100 and 50 Gy, respectively and that holds true for both the two successive seasons. Deaf (2007) and Taha (2009) came to the same conclusion that gamma radiation doses had the capacity to stimulate sugars synthesis in other aromatic plants.

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التأثير المرتبط للريزوبكتريا المنشطة لنمو النبات وأشعة جاما على النمو وإنتاجية الزيت الطيار لنبات الشمر

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تم تعريض بذور الشمر لجرعات من أشعة جاما صفر ، 50 ، 100 ، 150 جراي كما تم نقع البذور المشعة والغير مشعة في معلق بكتيري من *B. subtilis* ، *P. fluorescens* ، *Sinorhizobium* ، *Bradyrhizobium* لمدة 15 دقيقة. تم زراعة البذور المعاملة والغير معاملة في تربة طميية رملية خلال موسمين 2008 و 2009 وفي مرحلة تمام التزهير تم تسجيل الأوزان الطازجة والجافة لسيقان النباتات وفي مرحلة تمام النضج تم حصاد الثمار وتسجيل محصول البذوركما تم تقدير نسبة الزيت الطيار في بذور الشمر وتم حساب محصول الزيت الطيار. تم عمل تفريد لمكونات الزيت الطيار المستخاص من البذور الغير مشعة والمشعة بجرعة 100 جراي تحت عمليات التلقيح البكتيري المختلفة بإستخدام جهاز التحليل الكروماتوجرافي الغازي. هذا بالإضافة إلي أنه قد تم تقدير كل من المحتوي من السكريات الكلوروفيل في سيقان نبات الشمر. وقد أثبتت النتائج المتحصل عليها أن كل المعاملات المطبقة أمكنها تنشيط النمو النباتي حيث أنها أدت لزيادة الأوزان الطازجة والجافة للنباتات النامية ومن ثم زيادة محصول الثمار والبذوركما إزداد إنتاج الزيت الطيار نتيجة لزيادة محصول البذور إلا أنه لم يكن هناك تأثير علي مكونات الزيت الطيار كنتيجة للمعاملات المطبقة ولقد تفوقت جرعة الإشعاع 100 جراي والتلقيح بـ Bradyrhizobium في تحسين نمو النبات وتعظيم إنتاج الزيت الطيار خلال موسمي الدراسة.