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Rhizobium-Fenugreek Symbiosis Affected by Nitrogen Fertilizer Rates and Seeds Irradiation: A Field Experiment

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ABSTRACT

Seeds of fenugreek (*Trigonella foenum - graecum* L.) either irradiated (100 Gy) or non-irradiated were sown under clay soil conditions. Both seeds were inoculated with *Rhizobium* strains to assess the bacterial-plant symbiosis performance as affected by nitrogen fertilizer rates. Un-inoculated control was also included. ^{15}N labeled ammonium sulfate enriched with 5% atom excess was applied at 20 and 40 kg N fed^{-1}, in addition to non-fertilized treatment to trace and quantify portion of N derived from air (Ndfa).Vegetative growth parameters like shoot length, shoot and pod dry weights, 1000-seeds weight, and nodulation criteria (numbers and dry weight) were significantly enhanced by increasing N fertilizer rates, *Rhizobium* inoculation and irradiation dose. Raising N fertilizer rate had increased the growth attributes as well as nitrogen and phosphorus uptake by pods and shoots. *Rhizobium* inoculation improved the plant growth and compensated remarkable amount of nitrogen derived from air (Ndfa) to both plant organs. Fenugreek seeds exposed to gamma ray at low dose reflected positive significant response which translated into good growth attributes and nutrients uptake as well as effective *Rhizobium*-fenugreek symbiosis performance. This combined strategy as low cost effective and safe could be accepted to ensure remarkable fenugreek production with good quality under such given conditions.

Keywords: fenugreek, gamma ray, nitrogen rates, *Rhizobium*, symbiosis, yield

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum*) is a member of the Fabaceae (previously and better known as legumes). It has a multitude of uses including using the fresh or dried leaves, using the whole or ground seeds as a spice or the plant acts as an effective green manure to improve the soil components. In this respect, leaves and seeds are consumed in different countries around the world for different purposes such as medicinal uses (anti-diabetic, lowering blood sugar and cholesterol level, anti-cancer, anti-microbial, etc.), making food (stew with rice in Iran, flavor cheese in Switzerland, syrup and bitter run in Germany, mixed seed powder with flour for making flat bread in Egypt, curries, dyes, young seedlings eaten as a vegetable, etc.), roasted grain as coffee-substitute (in Africa), controlling insects in grain storages, perfume industries, and etc. Fenugreek can be a very useful legume crop for incorporation into short-term rotation and for hay and silage for livestock feed, for fixation of nitrogen in soil and its fertility (Sadeghzadeh-Ahari, *et al*. 2009).

Plant growth promotion via inoculation with microorganisms was proved elsewhere and accepted as an alternative source of plant nutrients. The responsible mechanism may include excretion of hormones, P-solubilizing activity, N_2 fixation and biological control or so called antagonistic activity (Arfaoui *et al*., 2006; Hussain *et al*., 2009; Deshwal *et al*., 2011; Gachande and Khansole, 2011). The supplement with N_2 via biological nitrogen fixation minimized the dependence on exogenous nitrogen fertilizers (Singh *et al*., 2011; Shahzad *et al*., 2012).

In this regard, *Rhizobium* has a beneficial role in improving plant growth as well as development of soil fertility (Shahzad *et al*., 2012; Deshwal and Chaubey, 2014). *Rhizobium* species which have six effective genera (Okazaki *et al*., 2004) may act as free-living or as nitrogen fixing endo-symbionts of legume host plants through root nodules or as associative microorganisms (Gothwal *et al*., 2008; Shamseldin *et al*., 2008). In addition, surrounding root rhizosphere includes many types of microorganisms and fungi or actinomycets that plays an important role in improving soil conditions (Gothwal *et al*., 2008). Rhizobia have ability to provide the host with N_2-fixed and benefits from the nutrients and energetic substances excreted by the host (Singh *et al*., 2011). Additionally, legumes itself are considered sources of nutrients that improved the nutrient-deficient soils and providing needed nutrients to humans and animals (Shahzad *et al*., 2012).

Low to mediate doses of gamma radiation found to be positively effective on growth parameters and yield of fenugreek crop (Hanafy and Akladious, 2018). Irradiation with low to mediate doses may be profitable for improving physiological and biochemical processes in irradiated plants as recognized earlier (Borzouei *et al*., 2013).

Interaction between gamma rays and water molecules can produce free radicals that can modify important components of plant cells depending on the irradiation dose (Wi *et al*., 2006). Indeed, free radical's generation acts as stress signals and trigger stress responses that may increase polyphenol acid content which had notable antioxidant properties (Fan *et al*., 2003).

Nitrogen is a limiting and crucial factor in crop production. This element may be applied at different rates either over or under the recommended dose N-fertilizers for fenugreek which affecting the productivity and profit margins to the farmers (Mehta *et al*., 2011). Due to the lake in information about the cost-effective nutrient management and needs to evaluate the impact of *Rhizobium*, this work aimed to trace the effects of rhizobium strains as most cheap and eco-friendly agent under different nitrogen fertilizer rates and gamma radiation on growth and productivity of fenugreek.

MATERIALS AND METHODS

Experimental layout

A field experiment was conducted on clay soil located at private farm (Badaway, Mansoura, Dakahleia Governorate, Egypt) with latitude of 31° 2' 16.5588" N and longitude 31° 22' 53.4828" E with elevation 10 m height over the sea level. Irradiated and non-irradiated seeds of fenugreek (*Trigonella foenum-graecum* L.) Giza 2 was sown on 2017, under drip irrigation system. The experimental design was split-split plot design within randomly complete block design (RCBD). Main plots were assigned to the three N rates and two inoculums were done in sub plots, while the two treatments of irradiation were occupied in the sub-sub plots. Hence, the total number was 3 levels (N) × 2 levels (inoculum) x 2 levels (irradiation) = 12 treatments and each treatment was replicated 3 times to give a total number of 36 experimental units. Each unit (plot) has an area of 42 m^2 (7 m long x 6 m width). Seeds were immersed with ten milliliters of broth media containing approximately 10^8 rhizobial growing cells, before seeding and this process was repeated after 7 days to ensure a sufficient *Rhizobium* population in the soil. After 55 days from seedling, samples of some fenugreek plants were harvested for nodules criteria estimation. After 120 days whole fenugreek plants were harvested to estimate the tested plant growth criteria, yield and NP uptake. All experimental data were subjected to ANOVA analysis and Duncan's multiple range test (DMRT) was followed for comparison between significant means at probability 0.05 using MSTAT-C program software version 1.42.

Mineral and bio-fertilization

Phosphorus and potassium fertilizers: Super-phosphate and potassium sulfate were added at basal rates recommended by Ministry of Agriculture at 150 kg P fed^{-1} and 60 kg K $fed^{-1}$for fenugreek plants, respectively.

Isotopic ^{15}N labeled fertilizer: Nitrogen was applied at 21 days after sowing in the form of labeled ammonium sulfate at the rates of 20 and 40 kg N fed^{-1} (5% ^{15}N atom excess). The labeled fertilizer was applied in micro-plot with 2 m^2 area.

Rhizobium inoculum: *Rhizobium meliloti* strain was applied in addition to the un-inoculated control. Used strain was kindly provided by Rhizobium production unit; Soil, Water and Environment Institute, Agricultural Research Center (ARC), Ministry of Agriculture, Giza, Egypt.

Seeds: Seeds of *Trigonella foenum-graecum* L. (Giza 2) were provided by Legume Crop Department, Institute of Field Crops Research, Agricultural Research Center (ARC), Ministry of Agriculture, Giza, Egypt. Seeds were separated into two groups one was subjected to gamma radiation at dose of 100 Gy and the other kept as non-irradiated ones. The source for gamma rays was cobalt-60 unit, National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority, Cairo, Egypt. Both of irradiated and non-irradiated seeds were dipped in *Rhizobium* cell suspension for 10 min and sterile distilled water (control), then dried overnight, and germinated on 1% water agar (WA) (10 seeds/plate).

Soil: the experimental soil located at Badaway village site was classified as clay soil according to its physic-chemical properties (Table 1). Physical and chemical analysis of the experimental soil was carried out according to Carter and Gregorich (2008).

$^{15}N/^{14}N$ ratio analysis

Plant samples were collected, dried and ground to very fine particles and subjected to analysis of $^{15}N/^{14}N$ ratio to determine $^{15}N\%$ atom excess using emission spectrometer model Fischer-NOI-6 PC. The following equation was applied to estimate the portion of N_2 fixed by inoculated plants (IAEA 2001):

Nitrogen derived from air (%Ndfa)

$$\%Ndfa = 100\,(1 - {}^{15}N\%\ \text{a.e. of inoculated plant} / {}^{15}N\%\ \text{a.e. of un-inoculated plant})$$

$$N_2\text{-fixed (kg fed}^{-1}) = \%Ndfa \times \text{total-N uptake by fixing crop}/100$$

Where, a.e. = ^{15}N atom excess

Table 1. Some physical and chemical properties of experimental soil.

Property	Value
Physical properties	
Sand (%)	12.2
Clay (%)	68.4
Silt (%)	19.4
Soil texture	Clay
Chemical properties	
pH (1:2.5)	7.9
E.C. dS m^{-1}	0.91
Total N (%)	0.44
Total P (%)	0.28
Total K (%)	0.39
Total Fe (%)	0.53
Total Mn (%)	0.02
Total Zn (%)	0.52
Total Cu (%)	0.03
Cation (meq L^{-1})	
Na^+	3.54
K^+	0.35
Ca^{++}	1.31
Mg^{++}	4.23
Anion (meq L^{-1})	
HCO_3^{--}	5.57
CO_3^-	-
Cl^-	2.3
SO_4^{--}	1.56

RESULTS AND DISCUSSION

Growth and yield parameters

Vegetative growth parameters such as shoot length, shoot fresh weight, pods fresh weight, 100-seeds weight, pods no $plant^{-1}$ were frequently affected by bacterial inoculation, gamma irradiation and nitrogen fertilizer rates.

In this respect, shoot length (Table 2), was tended to increase with increasing nitrogen fertilizer rates up to 40 kg fed^{-1} comparing to the unfertilized control. This holds true either under irradiated or the non-irradiated plants. Shoot length was positively affected by *Rhizobium* inoculation and exposure to gamma ray. Effective role of Rhizobium on shoot length was previously confirmed by Chaichi *et al*., (2015) who indicated that fenugreek plant height was significantly increased by *Rhizobium* inoculation over the un-inoculated control. This may be attributable to nitrogenase activity and synthesis of growth-promoting substances by N fixing bacteria. In the same time, Alnoaim and Hamad (2004) found that inoculation with bacteria in combination with chemical N fertilizer added at rate of 180 kg ha^{-1} (equal to 75 kg N fed^{-1}) achieved the highest plant growth attributes of rice. An explanation stated by Mehta *et al*., (2012) referred this phenomenon to the increase in root growth, and better nodulation. Enhancement of nutrient availability in the plant rhizosphere also induced an improvement of plant growth and pod formation (Mehta *et al*., 2012). Adequate to high bacterial cells as a result of exogenous inoculation may leads to a better symbiosis activities in rhizosphere which achieved remarkable amount of N fixed. Similar outputs were detected earlier by Purbey (2004).

Chaichi *et al*., (2015) found that most of the sole and integrated bio-fertilizers like *Azotobacter* + *Pseudumunas fluorescence* had positive effect on yield components of fenugreek. Their findings support those reported earlier by Ghosh and Mohluddln (2000). Increase in growth and yield attributes due to enhanced nutrient uptake will have direct and positive effect on seed, straw and biological yields of fenugreek (Godara *et al*., 2017; 2018).

Table 2. Shoot length (cm) of irradiated and non-irradiated fenugreek as affected by mineral nitrogen rates and rhizobium inoculation.

Nitrogen rates	Irradiated (B)			Non-irradiated		
kg fed^{-1} (A)	inoculated	Un-inoculated	Mean (A)	Inoculated	Un-inoculated	Mean (A)
0	21.0 c	16.0 d	18.5	18.0 c	15.0 c	16.5
20	49.0 b	47.0 b	48.0	38.0 ab	34.0 bc	36.0
40	63.0 a	55.0 a	59.0	44.0 a	41.0 a	42.5
Mean (B)	44.3	39.3	41.8	33.4	30.0	31.7
Mean (C)		38.9			34.7	

Means in the same column followed by the same letter are not significantly different at p≤ 0.05

Fenugreek treated with different N rates resulted in an increase in shoot dry weight with increasing fertilizer rate up to 40 kg N fed^{-1} (Table 3). This was true in case of both irradiated and non-irradiated plants. In this respect, shoot dry weight of irradiated crop was significantly higher than those of the non-irradiated ones. Also, the inoculated plants accumulated more dry matter yield of shoots comparing to the un-inoculated plants. This means that *Rhizobium*-fenugreek symbiosis was well responded to the gradient increase of nitrogen fertilizer and in the same time enhanced by irradiation. All tested factors were found to be significantly effective on accumulation of shoot dry matter yield.

Table 3. Shoot dry weight (kg fed^{-1}) of irradiated and non-irradiated fenugreek as affected by mineral nitrogen rates and rhizobium inoculation.

Nitrogen rates	Irradiated (B)			Non-irradiated		
kg fed^{-1}(A)	inoculated	Un-inoculated	Mean (A)	Inoculated	Un-inoculated	Mean (A)
0	5184.6 bc	4400.0 bc	4792.3	5005.6 c	3769.2 c	4387.4
20	6646.2 b	5015.4 b	5830.8	6323.1 b	4676.9 b	5500.0
40	8646.2 a	6830.8 a	7738.5	8092.3 a	5707.7 a	6900.0
Mean (B)	6825.7	5415.4	6120.6	6473.7	4717.9	5595.8
Mean (C)		6649.7			5066.7	

Means in the same column followed by the same letter are not significantly different at p≤ 0.05

Overall means reflected that increasing the N fertilizer rate added to the irradiated plants induced a relative increase in shoot dry weight by about 17.8% and 38.1% over the unfertilized control for 20 and 40 kg N fed^{-1}, respectively. In the same time, overall means of inoculation reflected an increase of shoot dry weight by about 20.7% over the un-inoculated ones. These relative increases were slightly higher in case of the non-irradiated plants recording 25.4% and 57.3% for the same sequences.

In this respect, inoculation induced relative increase in shoot dry weight of the non-irradiated plants by about 37.2% over the un-inoculated plants.

As shown in Table (4), *Rhizobium* inoculation significantly influenced pods dry weight as affected by different N fertilizer rates under irradiated and non-irradiated plants. Pods dry weight tended to increase with nitrogen fertilization comparing to the un-fertilized control. Irradiated and inoculated plants didn't reflect significant differences in pods dry weight between 20 and 40 kg N rates. Similar trend was noticed with inoculated but non-irradiated pants. In this regard, there was no significant difference between irradiated and non-irradiated plants.

Similarly, but to somewhat lower extent, was the case of the un-inoculated plants. In conclusion, pods dry weight was significantly positively affected by nitrogen fertilizer rates and rhizobial inoculation but it isn't the case for irradiation.

Table 4. Pods dry weight (kg fed^{-1}) of irradiated and non-irradiated fenugreek as affected by mineral nitrogen rates and rhizobium inoculation.

Nitrogen rates	Irradiated (B)			Non-irradiated		
kg fed^{-1} (A)	inoculated	Un-inoculated	Mean (A)	inoculated	Un-inoculated	Mean (A)
0	1.09 b	0.79 ab	0.94	1.16 ab	0.83 ab	0.99
20	1.37 a	0.98 ab	1.18	1.34 a	0.96 ab	1.15
40	1.47 a	1.19 a	1.33	1.44 a	1.13 a	1.28
Mean (B)	1.31	0.99	1.15	1.31	0.97	1.14
Mean (C)		1.31			0.98	

Means in the same column followed by the same letter are not significantly different at p≤ 0.05

In consistent, Meena *et al*., (2014) revealed that fenugreek plant height at harvest, dry matter accumulation plant^{-1}, number of branches plant^{-1}, number of nodules plant^{-1}, number of pods plant^{-1}, pod length, pod weight, number of seeds pod^{-1}, seed yield q ha^{-1} and straw yield were significantly positively affected by *Rhizobium* inoculation under different nitrogen fertilizer rates. They added that seed inoculation with *Rhizobium* + phosphate solubilizing bacteria (PSB) resulted in the maximum plant height, dry matter accumulation plant^{-1}, number of branches plant^{-1}, number of nodules plant^{-1}, number of pods plant^{-1}, pod length , pod weight, number of seeds pod^{-1}, seed yield q ha^{-1} ,straw yield q/ha as compared to control. Also, Abdul Hussein *et al*., (2013) found that rate of 90 kg N ha^{-1} was superior over 60 and 120 kg N ha^{-1} in increasing pods number plant^{-1}, seeds number pod^{-1}, and seed yield kg ha^{-1}.

The weight of 1000-seeds was enhanced by Rhizobium inoculation of either irradiated or non-irradiated plants (Table 5). Irradiation has a positive effect on enhancement of 100-seeds dry weight. Obviously, the dry weight of 1000-seeds was promoted by nitrogen fertilization and the highest values were detected with addition of 40 kg N fed^{-1} comparable to those treated with 20 kg N rate. This was true for irradiated and non-irradiated plants. Relatively, it increases by about 85% and 82% over the non-fertilized control for inoculated and un-inoculated irradiated plants while it was 101% and 27% for the same sequence under non-irradiation condition.

Table 5. 1000-seed weight (g) of irradiated and non-irradiated fenugreek as affected by mineral nitrogen rates and rhizobium inoculation.

Nitrogen rates	Irradiated (B)			Non-irradiated		
kg fed^{-1} (A)	inoculated	Un-inoculated	Mean (A)	inoculated	Un-inoculated	Mean (A)
0	15.8 c	13.5 c	14.7	11.3 c	11.7ab	11.5
20	23.6 b	20.2 ab	21.9	19.6 ab	12.4 ab	16.0
40	29.2 a	24.6 a	26.9	23.8 a	14.9 a	19.4
Mean	22.9	19.4	21.2	18.2	13.0	15.6
Mean (C)		20.6			16.2	

Means in the same column followed by the same letter are not significantly different at p≤ 0.05

Both of 1000-seeds weight and nodules numbers and dry weight plant^{-1} was found to be positively responded well to addition of 20 kg N ha^{-1} and inoculation with different strains of *Rhizobium meliloti* comparing to the untreated control (Singh and Patel, 2016). In this regard, integration between mineral fertilizer and *Rhizobium* bioinoculant may be considered as amenable and effective strategy that play a vital role in increasing and sustaining agricultural production (Jeyabal *et al*., 2000).

On line, Wierzbowska and Żuk-Gołaszewska (2014) found seed inoculation and increasing nitrogen rates more effective and led to significant differences in selected morphological parameters, such as the weight of selected plant organs and yield components. Similarly, the inoculated fenugreek with *Rhizobium meliloti* plus medium N rate had enhanced both the number of pods and seeds per plant. Raising the N fertilizer rate from 0.5 to 1.0 g N per pot significantly influenced morphological parameters (plant height, number of branches, stem weight) and yield components (thousand seed weight, seed weight). In this regard, they indicated that nitrogen fertilization contributed to a significant increase in thousand seed weight and seed weight (by 76.6% and 60%, respectively) as compared to the non-fertilized control.

Concerning the effect of irradiation, Hanafy and Akladious (2018) found that the progressive increase in growth and yield parameters of irradiated fenugreek plants was detected with gamma doses from 25 to 200 Gy, but dose 100 Gy was found to be the most effective one. Also, they recorded growth inhibition of irradiated plants exposed to 400 Gy below that of the un-irradiated control. In addition, it was explained earlier (Wi *et al*., 2007) that the interaction between gamma-rays and water molecules that produce free radicals which modified some of the important components of plant cells depending on the irradiation dose. In this respect, free radicals generation acts as stress signals and trigger stress responses that may increase polyphenol acid content which had notable antioxidant properties (Fan *et al*., 2003).

With respect to nitrogen rate, Mehta *et al*., (2011), recorded an increase in dry matter accumulation (DMA) per plant, crop growth rate (CGR), relative growth rate (RGR) and net assimilation rate (NAR) at all the crop growth stages with application of 20 kg N ha^{-1}. They attributed this phenomenon to early and sufficient availability of N to plants when biologically fixed N is not available to plants. It leads to better nutritional environment in the root zone for growth and development of plant. Similarly, was the case of grains and straw yields.

Nodulation

Nodule criteria were promoted by compatible strain of *Rhizobium meliloti* whereas the number and dry weight were increased in case of inoculated plants over those recorded with the un-inoculated one. Non-irradiated plants reflected lower nodule numbers and dry weight, except the non-fertilized control, than the irradiated plants. In the same way, both nodules number and dry weight were enhanced by nitrogen fertilization but there was no significant difference between the applied rates. It was obvious that the all tested factors have a positive significant role on enhancement and promotion of nodules formation on roots of fenugreek plants (Table 6).

Table 6. Nodules number and dry weight (g plant^{-1}) of irradiated and non-irradiated fenugreek as affected by mineral nitrogen rates and rhizobium inoculation.

Nitrogen rates	Irradiated						Non-irradiated					
kg fed^{-1}	inoculated		Un-inoculated		Mean		inoculated		Un-inoculated		Mean	
	No.	DW	No.	DW	No.	DW	No.	DW	No.	DW	No.	DW
0	16.2c	0.74c	9.3b	0.046d	12.8	0.39	14.1c	0.67c	8.7c	0.2c	11.4	0.44
20	55.7a	1.32a	16.5a	0.84a	36.1	1.08	38.8a	0.98a	19.7a	0.54a	29.3	0.76
40	56.4a	1.34a	15.8a	0.83a	36.1	1.09	43.6a	0.85b	21.9a	0.56a	32.8	0.71
Mean	42.8	1.13	13.9	0.57	28.3	0.85	32.2	0.83	16.8	0.43	24.5	0.63

Means in the same column followed by the same letter are not significantly different at p≤ 0.05

Rhizobial inoculants were found too be more beneficial in promoting the nodulation process comparing to the un-inoculated fenugreek plants (Badar *et al*., 2016). It seems that the enrichment of plant rhizosphere with pure cultures of *Rhizobium* significantly amplified the number of nodules and leads to increase the number of root nodules that raise the capability of nitrogen fixation (Badar *et al*., 2015 a&b). Similarly, fenugreek showed nodules on their roots with different wild and commercial Rhizobium strains, which indicates the compatibility between the plant and bacterial strains (Arafa *et al*., 2018). This finding proved that the nodulation process was strongly correlated to rhizobial strains where some of them may effectively possessed than others (Aliyu *et al*., 2013).

Nitrogen derived from air (Ndfa)

As well responded to *Rhizobium* inoculation, fenugreek plants had derived moderate portions and amounts of nitrogen from air (Ndfa) as fixed in root nodules and consequently derived by shoot and pods (Table 7). This portion of nitrogen was significantly positively affected by irradiation dose whereas both Ndfa by shoots and pods were higher in irradiated plants comparable to the non-irradiated ones. Percentages and absolute values of Ndfa were significantly higher in pods than shoots. Statistically, there was no significant difference between the two rates of fertilizer-N added to the inoculated plants. Nitrogen fixed and derived by pods of irradiated plants relatively surpassed those of the non-irradiated plants by about 25% when the mean average was considered.

Table 7. Nitrogen derived from air (percent and kg fed^{-1}) of irradiated and non-irradiated fenugreek as affected by mineral nitrogen rates and rhizobium inoculation.

Nitrogen rates kg fed^{-1}	Irradiated				Non-irradiated			
	Pods		Shoot		Pods		Shoot	
	%	Kg	%	kg	%	kg	%	kg
0	-	-	-	-	-	-	-	-
20	49.2a	166.0a	38.5a	31.0a	42.9a	128.8a	31.3a	20.3a
40	45.9a	158.7a	37.1a	35.4a	40.2a	130.0a	30.1a	22.7a
Mean	47.6	162.4	37.8	33.2	41.6	129.4	30.7	21.5

Means in the same column followed by the same letter are not significantly different at p≤ 0.05

Rhizobium cells numbers in the rhizosphere of fenugreek was increased with seeds inoculation which leads to more infection of roots and consequently increased amount of biologically fixed N. This possibly resulted in increased uptake of N which significantly influenced growth parameters of fenugreek (Mehta *et al*., 2011). In addition, they found that inoculation resulted in better root development, nodulation, nutrient availability resulting in vigorous plant growth and dry matter production and ultimately higher yield. Similar findings were recorded by Ali *et al* (2009) in fenugreek. In accordance with results of Wierzbowska and Żuk-Gołaszewska (2014), seed inoculation with *Rhizobium meliloti* increased the nitrogen content of pods by 41.3%. They recorded the highest nitrogen concentrations in the pods of inoculated plants that fertilized with a high nitrogen rate.

Nitrogen and phosphorus uptake

Nitrogen uptake by shoot and pods was increased with raising N fertilizer rate from 20 up to 40 kg N fed^{-1} (Table 8). *Rhizobium* inoculation led to enhance N uptake by both organs. Also, irradiation has a positive significant role in increasing nitrogen uptake by shoot and pods. More nitrogen was accumulated in pods than shoots. Except the effect of raising N rates on enhancement of P uptake by shot and pods, either inoculation or irradiation dose has no any significant role in this respect. P uptake by pods was slightly differed as compared to those uptaken by shoots.

Table 8. Nitrogen and phosphorus uptake (kg fed^{-1}) by shoot and pods of irradiated and non-irradiated fenugreek as affected by mineral nitrogen rates and rhizobium inoculation.

Nitrogen rates kg fed^{-1}	Irradiated						Non-irradiated					
	inoculated		Un-inoculated		Mean		inoculated		Un-inoculated		Mean	
	N	P	N	P	N	P	N	P	N	P	N	P
							Shoot					
0	58.1b	0.04b	43.4b	0.04a	50.8	0.04	48.9b	0.03b	36.7b	0.04b	42.8	0.03
20	80.6ab	0.07a	69.3ab	0.05a	74.9	0.06	64.9ab	0.08a	50.3a	0.09a	57.6	0.06
40	95.3a	0.07a	79.7a	0.06a	87.5	0.06	75.4a	0.09a	59.6a	0.10a	67.5	0.09
Mean	78.0	0.06	64.1	0.05	71.1	0.05	63.1	0.07	48.9	0.08	56.0	0.06
							Pods					
0	237.4b	0.09c	104.4c	0.08c	170.9	0.08	203.7b	0.07c	77.8c	0.08c	140.8	0.07
20	337.4a	0.22b	236.6b	0.17b	287.0	0.20	300.3ab	0.16b	217.4a	0.14b	258.9	0.15
40	345.7a	0.26a	258.5a	0.21a	302.1	0.24	323.5a	0.24a	239.2a	0.20a	281.4	0.22
Mean	306.8	0.19	199.8	0.15	220.0	0.17	275.8	0.16	178.1	0.14	227.0	0.15

Means in the same column followed by the same letter are not significantly different at p≤ 0.05

The present results are in agreement with those of Arafa *et al*., (2018), who found that fenugreek was well responded to rhizobial inoculation where N% and P % in fenugreek were enhanced as compared to the un-inoculated control. This reflected a positive effect on both plants fresh and dry matter which increased with increasing N levels. They added that higher increase in N and P uptake of plants treated with microbial inoculations suggest that a positive interaction exists between N and P uptake, root colonization, and growth promotion. The increased P absorption by plants might be due to a solubilizing effect of acidic exudates produced by the microbes prominently present in the rhizosphere and effective *Rhizobium* isolates (Yadav and Verma, 2014). In the same direction, increases of N and P content in grain and straw and total uptake N and P was probably due to more nitrogen fixation by the bacteria resulting in better utilization of all other nutrients by crop. These findings support those of (Dubey *et al*., 2012).

In response to increasing N rate, Mehta *et al*., (2011) recorded that application of 20 kg N ha^{-1} was found to be significant in improving NPK uptake over its lower dose (10 kg N ha^{-1}). They attributed the increase in uptake of N, P and K by crop with 20 kg N ha^{-1} to cumulative effect of increased yield and comparatively higher content of N, P and K in seed and straw than 10 kg N ha^{-1}. Nitrogen fertilization resulted in larger accumulation of nutrient from a fast growing root system. In relation to *Rhizobium* inoculation, fenugreek has accumulated more N and P nutrients over the un-inoculated plants (Mehta *et al*., 2011; Ali *et al*., 2009). Nitrogen availability could enhanced plant growth and reflecting more translocation of other nutrients to plant (Rizvil *et al*., 2013), as well as enhancing chlorophyll content which increases synthesis of food material and their distribution towards the pods (Jain *et al*., 2003). Also, increases the cation exchange capacity (CEC) of roots due to N fertilization, enabling them to absorb more nitrogen from the soil, thus N and, subsequently, P might have been utilized in greater quantities due to their abundant availability (Nadeem *et al*., 2004).

CONCLUSION

The present study recognized and confirmed the positive significant role of bacterial inoculation as most cheap supplementary agent in combination with low to moderate rate of nitrogen fertilizer in enhancing the growth and nutrients availability to fenugreek crop. Low dose of gamma ray had enhanced the vegetative growth and yield attributes and nutrients uptake. Rhizobium-fenugreek symbiosis processed well since it compensated remarkable amounts of nitrogen via biological N_2-fixation. This process was effectively processed under starter to moderate doses of mineral-N fertilizer. We recommend the recent management strategy to be applied under such given conditions. It could be certified as a low cost effective strategy and in the same time could help in preservation of environment via minimization of dependent on chemical fertilizers as sole way to increase crop productivity. Also, this strategy may assist in the improvement of fenugreek production as an important medicinal legume crop which has a useful uses for human health.

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تأثر العلاقة التكافلية بين الريزوبيوم والحلبة بمعدلات النيتروجين السمادي وتشعيع البذور: تجربة حقلية

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تمت زراعة بذور الحلبة سواء المشعة أو غير المشععة في الأرض الطينية تحت ظروف الحقل. لقحت البذور جميعها بعزلات من الريزوبيوم لتتبع أداء العلاقة التكافلية ما بين البكتيريا والنبات متأثرة بمعدلات النيتروجين السمادى المضاف. اشتملت التجربة أيضا على معاملة غير ملقحة. أضيف سماد سلفات الأمونيوم المرقم بالنيتروجين-15 بنسبة وفرة 5% وبمعدلات 20 و 40 كجم نيتروجين للفدان الى جانب معاملة شاهد غير مسمدة وذلك لتتبع وحساب حصة النيتروجين المستمد من الهواء الجوي (المثبت حيويا). تحسنت معنويا مقاييس النمو الخضري مثل أطوال السيقان ومحصول المادة الجافة للسوق والقرنات ووزن الألف حبة وكذلك تكوين العقد الجذرية (العدد والوزن الجاف)، وذلك بزيادة معدل اضافة النيتروجين والتلقيح بالريزوبيوم والجرعة الاشعاعية. أدت زيادة المعدل النيتروجيني الى زيادة مكونات النمو وكذلك امتصاص كلا من النيتروجين والفوسفور بواسطة السيقان والقرنات. التلقيح بالريزوبيوم أدى الى تحسن مقاييس النمو كما وفر جزء ملموس ومعتبر من النيتروجين المستمد من الهواء الجوي والمثبت في اجزاء النبات. بذور الحلبة التي تعرضت للإشعاع بجرعة منخفضة عكست استجابة معنوية ايجابية والتي ترجمت الى مكونات نمو وامتصاص المغذيات بصورة جيدة والى جانب أداء مؤثر للعلاقة التكافلية ما بين الحلبة والريزوبيوم. تلك الاستراتيجية المركبة قليلة الكلفة والآمنة يمكن قبولها لضمان انتاجية جيدة من الحلبة ذات الجودة وذلك في ظل مثل تلك الظروف التجريبية.

الكلمات المفتاحية: الحلبة، أشعة جاما، معدلات النيتروجين، الريزوبيوم، التكافل، الإنتاجية.