Indian Journal of Agricultural Sciences **87** (2): 234–39, February 2017/Article
https://doi.org/10.56093/ijas.v87i2.67636

Productivity and quality of fenugreek (*Trigonella foenum-graecum*.) as influenced by fertility levels and brassinosteroid

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Received: 29 September 2015; Accepted: 11 August 2016

ABSTRACT

A field study was conducted during *rabi* seasons of 2012-13 and 2013-14 to assess the effect of different fertility treatments and brassinosteroid on productivity and quality of fenugreek (*Trigonella foenum-graecum* L.). Results revealed that the significantly higher values of yield attributes, seed yield (2 245 kg/ha), haulm yield (5 236 kg/ha) and quality parameters (protein, sapogenin, diosgenin and galactomannan) were recorded with application of 100 % RDF over lower fertility levels. Dual inoculation of seed with *Rhizobium* + PSB gave significantly higher yield attributes, seed yield (2 085 kg/ha), contents and yields of protein (450.33 kg/ha), sapogenin (26.87 kg/ha), diosgenin (11.76 kg/ha) and galactomannan (593.69 kg/ha) over their sole application. Interaction of fertility levels and biofertilizers was also found significant in yield attributes and consequently in seed yield and yields of quality parameters. Application of 100 % RDF + dual inoculation, being on a par with 80 % RDF + dual inoculation, gave the highest seed yield (2 287 kg/ha), protein yield (513.52 kg/ha), sapogenin yield (30.32 kg/ha), diosgenin yield (13.21 kg/ha) and galactomannan yield (677.44 kg/ha). Foliar spray of brassinosteroid 0.50 ppm brought about significantly higher yield attributes, seed yield and quality parameters over water spray and BR 0.25 ppm, respectively. Thus, integration of 80 % RDF with dual inoculation and supplemented with foliar spray of brassinosteroid 0.50 ppm at 50 and 70 DAS is better for realizing higher yield and quality in fenugreek.

Key words: Biofertilizers, Brassinosteroid, Fenugreek, Fertility level, Productivity, Quality

Fenugreek (*Trigonella foenum-graecum* L.) belonging to sub-family papilionaceae of family fabaceae, is annual herbaceous seed spice crop of north India particularly of Rajasthan. The seed is mainly used as condiment and in the pharmaceutical industry, especially in preparation of ayurvedic medicines, while young plants are used as a vegetable and forage. Being a legume spice, it has high nutritive value and seed contain the steroidal substance, diosgenin, which is used as an oral contraceptive (Elujoba and Hardman 1985) and for production of corticosteroid. Mucilage (galactomannan) and oleoresins obtained from methi (Fenugreek) are also in large demand. In spite of great utility of fenugreek, a little attention has been paid to enhance its productivity with quality for remunerative and sustainable cultivation. Since, the soils in these areas are low in organic matter and poor in fertility status, and on account of a legume crop inadequate fertilization is in vogue without any supplement source of nutrition. Introduction of efficient strain of bioinoculants in such soil through seed inoculation may help in boosting up the production (Subba Rao *et al*. 2002). To sustain the land and crop productivity, judicious use of fertilizer with integration of biofertilizers is important (Bhunia *et al*. 2006). It has also been observed that in fenugreek synthesis and translocation of photosynthates into sink is very poor at later stages of crop growth besides poor flowering and fruit set. The plant bioregulator, brassinosteroid, play an important role as it enhances and regulates the various physiological processes and balance the source and sink thereby increase the productivity as well as quality. Considering these aspects, a study was carried out to assess the impact of fertility levels, biofertilizers and brassinosteroid in enhancing the productivity of fenugreek.

MATERIALS AND METHODS

A field experiment was carried out during *rabi* season of 2012-13 and 2013-14 on sandy loam soil at ICAR-National Research Centre on Seed Spices, Ajmer (Rajasthan), situated at 460.2 m above mean sea level on latitude 26°22’ N and longitude 74°36’E and is characterized by hot semi-arid climate. The annual rainfall is mainly confined to three months (July- September) and mean precipitation is about 532 mm. The soils of the experimental site was sandy loam with a pH 8.03 having low organic carbon (0.28 %), low available nitrogen (151.3 kg/ha), low available phosphorus (7.8 kg/ha) and medium available potassium (197.1 kg/ha). The OC% was determined by the method Walkley and Black

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(1947), whereas available N, P and K were determined by the methods suggested by Subbiah and Asija (1956), Olsen *et al*. (1954) and Jackson (1973), respectively. The experiment was laid out in split plot design comprising three levels of fertility (F_1-60 % RDF, F_2-80 % RDF and F_3-100 % RDF) and three biofertilizer inoculation (B_1-*Rhizobium*, B_2-PSB and B_3- *Rhizobium* + PSB) as main plot and three concentrations of brassinosteroid (G_0- water spray, G_1-BR 0.25 ppm and G_2-BR 0.50 ppm) as sub-plot treatment replicated thrice. RDF comprised of 40 kg N + 40 kg P_2O_5 and 10 kg S/ ha. Sowing of fenugreek (RMt-143) using 20 kg seed/ha was done at 30 cm line to line spacing and plant to plant distance of 10 cm was maintained. Seed treatment with *Rhizobium meliloti,* PSB and combination of both was done as per treatment before sowing. Full dose of nitrogen, phosphorus and sulphur was provided at the time of sowing. The nitrogen, phosphorus and sulphur were supplied through urea, DAP and gypsum, respectively. Brassinosteroid was sprayed twice at 50 and 70 DAS as per treatment under study. Five plants were selected randomly from each plot to count number of pods/plant, pod length, number of seeds/pod and seed yield plant. Observation of yield, nutrient content and quality parameters were recorded to evaluate the effect of treatments, nitrogen in seed was analyzed as Nesseler's reagent colorimetric method. Protein content of seed was recorded by multiplying factor 6.25 to nitrogen content in seed, sapogenin and diosgenin content of the seed was determined as per methodology adopted by Baccau *et al.* (1977) and Uematsu *et al*. (2000) and galactomannan content of seed was estimated as per method suggested by Rathore *et al*. (2013). Yields of these quality parameters were determined by multiplying their contents in seed with seed yield. The statistical analysis was done as per procedure suggested by Panse and Sukhatme (1985).

RESULTS AND DISCUSSION

Yield attributes

It is discernible from the data (Table 1) that different yield attributes increased significantly with increase in fertility level from 60 to 100% RDF. The maximum number of pods/plant, seeds/pod, pod length, 1 000-seed weight and seed yield/plant was recorded with application of 100% RDF. The increase in pods/plant, pod length, seeds/pod, 1 000-seed weight and seed yield/plant with 100% RDF was in order of 13.7 and 4.5, 13.1 and 4.8, 16.0 and 7.0, 11.3 and 4.7, 33.1 and 10.0%, respectively, over 60 and 80% RDF. This might be due to early and abundant availability of nutrients with 100% RDF which resulted in higher growth and yield attributes. The results corroborate with findings of Singh and Agarwal (2005), Kumar *et al*. (2009) and Singh *et al.* (2010).

Table 1 Yield attributes and yields of fenugreek as influenced by fertility levels, biofertilizers and brassinosteroid

Treatments	Pods/ plant	Pod length (cm)	Seeds/ pods	Wt. of seeds/pod (g)	1 000-seed weight (g)	Seed yield / plant (g)	Seed yield (kg/ ha)	Haulm yield (kg/ ha)	Biological yield (kg/ha)	Harvest index (%)
Fertility level										
F_1: 60 % RDF	31.98	10.48	13.59	0.178	13.28	5.594	1659	3934	5593	29.71
F_2: 80 % RDF	34.80	11.36	14.67	0.201	14.11	6.771	2000	4677	6678	29.99
F_3: 100 % RDF	36.37	12.16	15.37	0.220	14.78	7.445	2245	5236	7480	30.04
SEm ±	0.35	0.12	0.13	0.002	0.14	0.074	24	62	81	0.18
CD (P=0.05)	1.00	0.35	0.39	0.007	0.39	0.214	69	178	234	NS
Biofertilizers										
B_1: *Rhizobium*	33.76	11.09	14.31	0.198	13.91	6.497	1934	4597	6532	29.60
B_2: PSB	33.43	10.78	13.98	0.192	13.58	6.293	1885	4308	6193	30.46
B_3: *Rhizob.*+PSB	35.96	12.13	15.33	0.210	14.68	7.020	2085	4941	7026	29.68
SEm ±	0.35	0.12	0.13	0.002	0.14	0.074	24	62	81	0.18
CD (P=0.05)	1.00	0.35	0.39	0.007	0.39	0.214	69	178	234	0.51
Interaction (FXB)	Sig	Sig	Sig	Sig	NS	Sig	Sig	Sig	Sig	NS
Brassinosteroid										
G_0: Water spray	32.98	10.79	13.84	0.185	13.79	6.091	1831	4320	6151	29.80
G_1: BR 0.25 ppm	34.46	11.37	14.60	0.201	14.06	6.654	1975	4636	6611	29.89
G_2: BR 0.50 ppm	35.72	11.84	15.18	0.213	14.31	7.065	2098	4891	6989	30.04
SEm ±	0.29	0.10	0.12	0.002	0.10	0.060	12	42	50	0.14
CD (P=0.05)	0.83	0.27	0.33	0.006	0.28	0.169	34	118	142	NS
Interactions (F×G, B×G and F×B×G)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Further appraisal of data (Table 1) reveals that combined application of both *Rhizobium* and PSB was found significantly better over their sole application with regard to yield attributes; however, sole *Rhizobium* was found on a par with PSB. Dual inoculation produced 6.5 and 7.6, 7.2 and 9.7, 5.6 and 8.2, 8.0 and 11.6% more pods/plant, seeds/pod, 1 000-seed weight and seed yield/plant, respectively over sole *Rhizobium* and PSB. *Rhizobium* and PSB improves the N and P availability of soil which are major plant nutrients and combined inoculation of both N_2 fixer and PSB benefits plants than either group of organisms alone and may have an added advantage. Furthermore, some of the bacteria involved might be interacting on more metabolic levels, i.e. P solublizer may also be auxin, IAA and gibberellins producer and N_2 fixer may also solublize P. Thus, the increased availability of not only N and P but growth hormones also stimulate plant metabolism which resulted in better yield attributes. These results are in accordance with the findings of Purbey and Sen (2007) and Mehta *et al.* (2012). Interaction effect between fertility levels and biofertilizers in respect of pods/plant, seeds/pod, weight of seeds/pod and seed yield/plant were observed significant (Table 2). The maximum values of aforesaid yield attributes were recorded under 100% RDF + dual inoculation which were found on a par with 80% RDF and dual inoculation. Statistically equally good performance of combination of 80% RDF + dual inoculation was might be due to relatively better bacterial activity at lower fertility level. It is well established that N fixation decreases at higher levels of nitrogen.

Foliar spray of brassinostroid (BR) @ 0.50 ppm resulted in significantly higher yield attributes over water spray and BR 0.25 ppm. Spray of BR 0.50 ppm gave 8.3 and 3.7, 9.7 and 4.0, 3.8 and 1.8, 16.0 and 6.2% higher pods/plant, seeds/pod, 1 000-seed weight and seed yield/plant over water spray and BR 0.25 ppm, respectively. Improvement in yield attributes ascribed to association of brassinosteroid with enhanced photosynthetic efficiency and transport of photosynthates to reproductive parts (Mandava 1988).These results are in close accordance with Farahat (2002) and Bera and Pramanik (2013).

Yield: It is apparent from the data presented (Table 1) that seed and haulm yield increased significantly with each successive higher fertility level from 60 to 100% RDF. The highest seed, haulm and biological yields of fenugreek were obtained with 100% RDF. Application of 100% RDF produced 35.3 and 12.2% higher seed yield over 60 and 80% RDF, respectively. The corresponding increases in haulm yield were 33.1 and 11.9%. However, the harvest index was not influenced with fertility level. Adequate nutrient availability at 100% RDF favoured growth and development of plant and thus increased growth and yield attributes which ultimately increased seed and haulm yield of fenugreek over 60 and 80% RDF. Increase in seed, haulm and biological yield with 100% RDF was also reported by Singh and Agarwal (2004), Kumar *et al*. (2009) and Mehta *et al*. (2012).

Table 2 Yield attributes as influenced by interaction effect between fertility levels and biofertilizers (pooled of two years)

Treatment	Pods/plant			Pod length (cm)			Seed weight/pod			Number of seeds/pod			Seed yield/plant		
(Biofertilizers/fertility level)	Rhizo.	PSB	Rhizo.+ PSB	Rhizo.	PSB	Rhizo.+ PSB	Rhizo.	PSB	Rhizo.+ PSB	Rhizo.	PSB	Rhizo.+ PSB	Rhizo.	PSB	Rhizo.+ PSB
60 % RDF	31.15	30.42	34.37	10.57	10.03	10.84	0.183	0.174	0.178	13.68	13.14	13.94	5.658	5.385	5.738
80 % RDF	34.56	33.24	36.62	10.86	10.50	12.74	0.195	0.184	0.224	14.17	13.84	16.00	6.477	6.212	7.624
100 % RDF	35.58	36.63	36.89	11.85	11.80	12.84	0.215	0.217	0.229	15.07	14.97	16.06	7.356	7.282	7.697
SEm ±		0.60			0.21			0.004			0.23			0.129	
CD (P=0.05)		1.74			0.61			0.011			0.67			0.371	

Combined inoculation of fenugreek seed with *Rhizobium* and PSB exhibited 7.8 and 10.6% higher seed yield over sole application of *Rhizobium* and PSB, respectively. The corresponding increases in haulm yield were 7.5 and 14.7%. However, both inoculants were remained on a par with each other w.r.t. yields. Synergistic effect of *Rhizobium* and PSB might have increased the growth and yield attributes which increased seed, haulm and biological yields of fenugreek due to higher nitrogenase activity and available P status of soil. These results corroborated with the findings of Jat and Shaktawat (2001) and Ali *et al*. (2009). Interaction effect between fertility levels and biofertilizers in respect of seed, haulm and biological yields were observed significant (Table 3). Application of 100% RDF + dual inoculation, being on a par with 80% RDF + dual inoculation, gave the highest seed, haulm and biological yield.

Brassinosteroid significantly influenced the seed, haulm and biological yield. However, harvest index was remained unaffected due to brassinosteroid. Foliar spray of BR 0.50 ppm produced 14.6 and 6.2% higher seed yield and 13.2 and 5.5% higher haulm yield over water spray and BR 0.25 ppm, respectively. These results were in conformity with the findings of Purbey and Sen (2005) and Singh *et al.* (2010).

Quality parameters

Protein content and yield: It is discernible from the data (Table 4) that protein content in seed and protein yield increased significantly with increase in each successive higher fertility level from 60 to 100% RDF. 100% RDF led to highest protein content (21.86%) and protein yield (491.63 kg/ha) which were significantly higher over, 60% RDF by 12.0 and 51.1 % and 80% RDF by 4.9 and 17.4%,

Table 3 Seed, haulm and biological yields (kg/ha) as influenced by interaction effect between fertility levels and biofertilizers

Treatment (Biofertilizers /fertility level)	Seed yield			Haulm yield			Biological yield		
	Rhizo.	PSB	Rhizo.+ PSB	Rhizo.	PSB	Rhizo.+ PSB	Rhizo.	PSB	Rhizo.+ PSB
60 % RDF	1672	1609	1697	4045	3696	4060	5717	5305	5757
80 % RDF	1898	1833	2270	4482	4177	5373	6379	6010	7643
100 % RDF	2233	2214	2287	5266	5050	5391	7499	7264	7678
SEm ±		42			107			141	
CD (P=0.05)		120			308			406	

Table 4 Quality parameters contents (%) and their yields (kg/ha) as influenced by fertility levels, biofertilizers and brassinosteroid

Treatment	Protein		Sapogenin		Diosgenin		Galactomannan	
	Content	Yield	Content	Yield	Content	Yield	Content	Yield
Fertility levels								
F_1: 60 % RDF	19.53	325.28	1.162	19.36	0.504	8.40	25.58	425.73
F_2: 80 % RDF	20.84	418.72	1.239	24.88	0.539	10.84	27.12	546.26
F_3: 100 % RDF	21.86	491.63	1.300	29.24	0.567	12.76	28.50	640.70
SEm ±	0.20	6.67	0.013	0.34	0.006	0.18	0.31	8.87
CD (P=0.05)	0.57	19.22	0.037	0.99	0.018	0.52	0.89	25.55
Biofertilizers								
B_1: *Rhizobium*	20.94	407.19	1.221	23.76	0.528	10.30	26.83	521.98
B_2: PSB	19.85	378.12	1.198	22.84	0.521	9.94	26.17	497.02
B_3: *Rhizob.*+PSB	21.44	450.33	1.283	26.87	0.561	11.76	28.20	593.69
SEm ±	0.20	6.67	0.013	0.34	0.006	0.18	0.31	8.87
CD (P=0.05)	0.57	19.22	0.037	0.99	0.018	0.52	0.89	25.55
Interaction (FXB)	NS	Sig	NS	Sig	NS	Sig	NS	Sig
Brassinosteroid								
G_0: Water spray	19.87	366.26	1.183	21.82	0.508	9.38	26.05	481.28
G_1: BR 0.25 ppm	20.86	415.54	1.237	24.61	0.538	10.73	27.11	539.90
G_2: BR 0.50 ppm	21.51	453.84	1.282	27.05	0.564	11.90	28.04	591.51
SEm ±	0.15	3.65	0.011	0.27	0.006	0.11	0.22	5.34
CD (P=0.05)	0.44	10.28	0.030	0.77	0.016	0.31	0.61	15.05
Interactions (F×G, B×G and F×B×G)	NS	NS	NS	NS	NS	NS	NS	NS

Table 5 Quality parameters' yield (kg/ha) as influenced by interaction effect between fertility levels and biofertilizers

Treatment (Biofertilizers/ fertility level)	Protein yield			Sapogenin yield			Diosgenin yield			Galactomannan yield		
	Rhizo.	PSB	Rhizo.+PSB	Rhizo.	PSB	Rhizo.+PSB	Rhizo.	PSB	Rhizo.+PSB	Rhizo.	PSB	Rhizo.+PSB
60 % RDF	337.49	296.23	342.13	19.31	17.78	20.97	8.35	7.73	9.12	432.68	404.38	440.13
80 % RDF	399.58	361.24	495.35	23.39	21.91	29.33	10.09	9.48	12.94	503.48	471.81	663.51
100 % RDF	484.49	476.88	513.52	28.58	28.84	30.32	12.46	12.62	13.21	629.78	614.87	677.44
SEm ±		11.55			0.59			0.32			15.37	
CD (P=0.05)		33.29			1.71			0.91			44.26	

respectively. It might be due to the fact that at higher fertility level there is more availability of N and S, which are constituents of protein. Similar findings were reported by Jat and Sharma (2000), Choudhary (2006) and Singh *et al.* (2002). Co inoculation of *Rhizobium* and PSB, being on a par with sole *Rhizobium*, enhanced protein content of seed significantly over sole PSB. However, significantly highest protein yield was recorded with coinoculation over their individual application. Sole *Rhizobium* also proved its superiority over sole PSB with regards to protein content and yield. Interaction effect of fertility levels and biofertilizers was also found significant in case of protein yield, maximum protein yield was recorded at a combination of 100% RDF + coinoculation, which was on a par with 100% RDF + *Rhizobium* and 80% RDF + coinoculation (Table 5). Brassinosteroid significantly influenced the protein content and yield. The maximum protein content and protein yield were obtained from foliar spray of BR 0.50 ppm, which produced 8.3 and 3.1% higher protein content 23.9 and 9.2% higher protein yield over water spray and BR 0.25 ppm, respectively. This might be due to the fact that brassinosteroid influences a number of enzymes involved in plant metabolisms primarily in synthesis of protein. Higher protein content and yield as a result of brassinosteroid sprays was also reported by Purbey and Sen (2007) and Singh *et al.* (2010).

Sapogenin content and yield: Sapogenin content in seed and its yield increased significantly with increase in each successive fertility level up to 100 % RDF. 100 % RDF produced maximum sapogenin content (1.30%) and sapogenin yield (29.24 kg/ha) which were significantly higher over 60 % RDF by 11.9 and 51.0 % and 80 % RDF by 4.9 and 17.5%, respectively. This can be ascribed to different structural and functional roles of nitrogen, phosphorus and sulphur at cellular level that have influenced synthesis of metabolites like sapogenin. Similar findings have also been reported by Singh *et al.* (2010). Coinoculation of *Rhizobium* and PSB enhanced sapogenin content and yield of significantly over their individual application. Sole *Rhizobium* also found superior over sole PSB with regards to sapogenin yield. Interaction effect of fertility levels and biofertilizers was also found significant with regard to sapogenin yield, maximum sapogenin yield was recorded at a combination of 100% RDF + coinoculation, which was on a par with 100% RDF + *Rhizobium* and 80% RDF + coinoculation. It might be due to greater availability of N and P which resulted in greater availability of metabolites to seed might have facilitated greater conversion of metabolites into steroids. Sapogenin content and its yield were also influenced significantly due to foliar spray of brassinosteroid (BR). The maximum sapogenin content and sapogenin yield were obtained from foliar spray of BR 0.50 ppm, which produced 8.4 and 3.7% higher sapogenin content 24.0 and 9.9% higher sapogenin yield over water spray and BR 0.25 ppm, respectively. These results are in close conformity with findings of Purbey and Sen (2007).

Diosgenin content and yield: It is obvious from the quality data presented (Table 4) that diosgenin content and its yield were also varied significantly with fertility levels.100% RDF produced maximum diosgenin content (0.567%) and diosgenin yield (12.76 kg/ha) which were significantly higher over 60% RDF by 12.5 and 51.9 % and 80 % RDF by 5.2 and 17.7%, respectively. It might be due to different structural and functional roles of nitrogen, phosphorus and sulphur at cellular level that have influenced synthesis of metabolites like diosgenin. These results corroborated with the findings of Singh *et al.* (2010). Coinoculation of seed with *Rhizobium* and PSB increased diosgenin content and yield significantly over their sole application. Further, sole *Rhizobium* had proved its superiority over sole PSB with regards to diosgenin yield. Moreover, interaction effect of fertility levels and biofertilizers was found significant with regard to diosgenin yield, maximum diosgenin yield was recorded at a combination of 100% RDF + coinoculation, which was on a par with 80% RDF + coinoculation (Table 5). Diosgenin content and its yield were also influenced significantly due to foliar spray of brassinosteroid (BR). The maximum diosgenin content (0.564%) and diosgenin yield (11.90 kg/ha) were obtained from foliar spray of BR 0.50 ppm, which produced 10.9 and 4.7% higher diosgenin content 26.9 and 10.9% higher diosgenin yield over water spray and BR 0.25 ppm, respectively. These results are in close conformity with findings of Purbey and Sen (2007) and Singh *et al.* (2010).

Galactomannan content and yield: It is evident from the data (Table 4) that galactomannan content and yield were increased significantly with increase in successive fertility level from 60 to 100% RDF. The highest galactomannan content (28.5%) and its yield (640.70kg/ha) were recorded under 100% RDF, which were significantly higher over that recorded at 60% RDF by 11.4 and 50.5% and 80 % RDF by 5.1 and 17.3 %, respectively. These results corroborated with the findings of Purbey and Sen (2007). Microbial inoculation substantially influenced content and yield of galactomannan. Coinoculation of seed with *Rhizobium* and PSB proved its superiority by significantly increasing galactomannan content and yield over their sole application. Moreover, interaction effect of fertility levels and biofertilizers was found significant with regard to galactomannan yield, maximum galactomannan yield was obtained from a combination of 100 % RDF + coinoculation, which was on a par with 80 % RDF + coinoculation (Table 5). Galactomannan content and its yield were also influenced significantly due to foliar spray of brassinosteroid (BR). The significantly highest galactomannan content (28.04%) and its yield (591.51kg/ha) were obtained from foliar spray of BR 0.50 ppm, which were 7.6 and 22.9% higher over water spray and 3.4 and 9.6 % higher over BR 0.25 ppm, respectively. This might be due to the fact that brasinosteroids are known to promote the metabolism of assimilates/ food material by increasing the various enzymatic activities. These results are in close conformity with those reported by Singh *et al.* (2010).

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