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Influence of nitrogen fixing and phosphorus solubilizing bacteria inoculation on fenugreek symbiotic properties, growth and yield

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***Abstract*— *A field experiment was conducted for two successive seasons 2012/2013 and 2013/2014 with four replicates at the experimental farm, Faculty of Agriculture, Omdurman Islamic University to determine the effect of inoculation and co-inoculation of symbiotic nitrogen fixing and phosphate solubilizing bacteria on fenugreek (Trigonella foenum-graecum L.) growth and yield. Fenugreek plant variety Kodab was used. Two bacterial strains Sinorhizobium meliloti strain TAL380 and Bacillus megatherium var. phosphaticum strain (BMP), each alone or in combination. Control plants were kept for comparison. The following measurements were taken per plant: number of nodules, dry weight of nodules, shoots and roots, plant height, pod length, number of pods/plant, number of seeds/pod, 1000 seed weight and yield. Results showed that inoculation with TAL380 and BMP each alone significantly increased nodulation and nodule dry weight compared to the control. Inoculation with BMP significantly increased root dry weight, when inoculation with TAL380 significantly increased shoot dry weight and plant height, co-inoculation significantly increased root and shoot dry weight compared to the control. The two bacterial inoculations each alone or in combination significantly increased number of pods and insignificantly increased number of seeds per pod and 1000 seed weight at both seasons. There was no effect of inoculation with bacteria on pod length. Co-inoculation significantly increased fenugreek seed yield at both seasons compared to the control.*

***Keywords*— Biofertilizers, nitrogen fixation, phosphorus solubilization.**

I. INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is a dicotyledonous plant belongs to family *Leguminoseae* (*Fabaceae*). It is native of Mediterranean region and grown as a spice (seeds and leaves) in most part of the world, it is also used to treat a variety of health problems [1, 2]. Fenugreek is symbiotically associated with the rhizobia and this requires active nitrogen fixation and the interaction plays a key role in the agricultural crop production. It can fix atmospheric nitrogen symbiotically in the range of 40-138Kg/ha [3].

Nitrogen is the critical limiting element for growth of most plants due to its unavailability [4]. Plants need nitrogen more than any other nutrient since it is an important constituent of proteins, enzymes, chlorophyll, and growth regulators [5]. Phosphorus is one of the most limiting nutrients for plant growth and phosphorus fertilization is a major input in crop production [6]. Phosphorus deficiency has a detrimental effect on legumes nodulation [7]. Successful production systems based on legumes therefore, requires P inputs [8] either from soil reserves or from added fertilizer [9].

Biofertilizers naturally activate the soil borne microorganisms to restore soil natural and protect it against drought and soil disease and therefore stimulate plant growth [10]. Biofertilizers include nitrogen fixing, phosphate solubilizing and plant growth promoting microorganism [11]. Using of biofertilizers that certain different microbial strain led to decrease the use of agro chemicals for human safety [12]. Number of soil borne bacteria had been used as a biofertilizers to promote plant growth such as: *Pseudomonas*, *Bacillus*, *Enterobacter*, *Azospirillum* and *Rhizobium* etc. they called rhizobacteria because they are related to rhizosphere of the plant. Rhizobacteria are bacteria that aggressively colonize the plant roots [13].

Rhizobium belongs to a well known group of bacteria that act as primary fixer of nitrogen in symbiotic relationship with legume plants [14]. A previous study showed that simultaneous infection with rhizobia and rhizosphere bacteria increases nodulation and growth in a wide variety of legumes [15]. It is possible to increase agricultural productivity and, eliminate or decrease the use of chemical fertilizers and pesticides even in marginal soils when the formulation contains different PGPB [16]. However, inoculation with a compatible strain of rhizobia was found to enhance nodulation, dry weight of nodules, nitrogen fixation and yield of alfalfa (*Medicago sativa*), Fenugreek

(*Trigonella foenum-graecum*), cluster bean (*Cyamopsis tetragonoloba*), field pea (*Pisum sativum*) and common bean (*Phaseolus vulgaris*) grown in dry land. It was concluded that, the productivity of leguminous crops in dry land could be improved by *Rhizobium* inoculation [17].

Phosphorus solubilizing bacteria mainly *Bacillus*, *Pseudomonas* and *Enterobacter* are very effective for increasing the plant available P in soil as well as the growth and yield of crops. So, exploitation of phosphate solubilizing bacteria through biofertilization has enormous potential for making use of ever increasing fixed P in the soil [18].

Co-inoculation with *Rhizobium* and *Bacillus megatherium* var. *phosphatcum* PSB increased pigeon pea nodulation, nodule dry weight, shoot dry weight and shoot nitrogen and phosphorus content, compared to un-inoculated control [19]. The co-inoculation with *Rhizobium* and PSB increased faba bean yield [20], chickpea yield [21] compared to single-inoculation with *Rhizobium*. Generally, co-inoculation can increase nodulation and improve grain yield, phosphorus and nitrogen uptake by the plants. The objective of the study was to determine the effect of inoculation and co-inoculation of symbiotic nitrogen fixing and phosphate solubilizing bacteria on fenugreek growth and yield.

II. MATERIAL AND METHODS

A field experiment was conducted at the experimental farm, Faculty of Agriculture, Omdurman Islamic University, Sudan, (Latitude 15° 34´ N and longitude 32° 34´ E), for two successive seasons 2012/2013 and 2013/2014 with four replicates. In this experiment, fenugreek plant variety Kodab was used. Two bacterial strains were obtained from Bio-pesticides and Bio-fertilizers Department, Environment, Natural Resources and Desertification Research Institute (ENDRI), National Center for Research (NCR), Khartoum, Sudan. *Sinorhizobium meliloti* strain TAL380 and *Bacillus megatherium* var. *phosphaticum* strain (BMP), each alone or in combination. Control plants were kept for comparison.

The land was prepared by deep ploughing, harrowing then leveling and ridging. The land was then divided into plots 5 x 4m each. Three to four seeds were placed in a hole on the top of the ridge with 15cm spacing (between holes) and 70cm (between ridges). Plots were immediately irrigated after sowing and then subsequently irrigated at 7-10 days intervals.

The first sampling was carried out after 6 weeks after sowing (WAS), and then sampling was done every two weeks, for 10 weeks. During each sampling, four plants from each plot were carefully dug out of the soil with their roots. Plants representing each treatment in each plot were placed in a paper bag and immediately taken to the laboratory for the following measurements per plant: number of nodules, dry weight of nodules, shoots and roots (after oven drying at 80°C for 48 hours). Plant height (cm) was measured at 10 WAS. Each plot was harvested separately by cutting the plants just above soil level. Five pods were taken randomly from each plot to measure pod length (cm). Pods of 5 plants from each plot were counted for number of pods/plant. Seeds of 5 pods from each plot were counted to measure number of seeds/pod. Plants were then threshed on a large mat, then collected and weighed to determine yield of each plot, 1000 seeds were counted and weighed for 1000 seed weight.

Analysis of variance (ANOVA) was used to determine the effect of treatments on the measured parameters. Least significance difference was used to compare between means, significance was accepted at $P \leq 0.05$ [22].

III. RESULTS

1, Nodule formation

Table 1 show the effect of inoculation by *Sinorhizobium meliloti* strain (TAL380) and *Bacillus megatherium* var. *phosphaticum* strain (BMP) each alone and in combination on fenugreek nodule formation in first and second season. There was poor nodulation on the control plants. Inoculation with TAL380 significantly ($P \leq 0.05$) increased nodulation compared to the control at both seasons except at 6 and 10 WAS in the first season where the increment was insignificant. Inoculation with BMP significantly ($P \leq 0.05$) increased nodulation at 6 and 8 WAS in the second season compared to the control.

2, Nodule dry weight

From results, at the first season, inoculation with TAL380 significantly ($P \leq 0.05$) increased nodule dry weight at 8 WAS compared to the control (Table 2). Inoculation with BMP significantly ($P \leq 0.05$) increased nodule dry weight at 10 WAS compared to control and other treatments. At the second season, inoculation with TAL380 gave the highest significant ($P \leq 0.05$) nodule dry weight at 10 WAS, followed by BMP.

3, Root dry weight

Results in table 3 shows that at the first season inoculation with BMP significantly ($P \leq 0.05$) increased root dry weight at 8 and 10 WAS compared to the control. At the second season, the highest root dry weight significant ($P \leq 0.05$) increment was obtained by co-inoculation at 8 WAS, followed by TAL380 and BMP, respectively.

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4, Shoot dry weight

At the first season, inoculation with BMP alone or in combination with TAL380 insignificantly increased fenugreek shoot dry weight (Table 4). At the second season, inoculation with TAL380 significantly (P≤0.05) increased shoot dry weight at all observation times, followed by co-inoculation at 8 and 10 WAS, when inoculation with BMP was superior at 8 WAS.

5, Plant height

Inoculation with TAL380 significantly (P≤0.05) increased plant height at the second season (Table 5). At both seasons, BMP increased plant height insignificantly.

6, Pod number

Bacterial inoculations significantly (P≤0.05) increased number of pods at both seasons (Table 5). Inoculation BMP gave the highest pod number followed by co-inoculation and TAL380, respectively.

7, Pod length

There was no effect of inoculation with bacteria on pod length (Table 5).

8, Number of seeds per pod

From results in table 6, both bacterial strains alone or in combination increased number of seeds per pod insignificantly at the two seasons compared to un-inoculated control. Overall mean of both seasons indicate that inoculation with BMP gave the highest number of seeds per pod, followed by co-inoculation.

9, 1000 seeds weight

Inoculation with both bacterial strains alone or in combination increased the weight of 1000 insignificantly at the two seasons compared to the control (Table 6). Overall mean of both seasons indicate that co-inoculation with TAL380+BMP gave the highest 1000 seeds weight, followed by inoculation with BMP.

10, Yield

All treatments significantly (P≤0.05) increased fenugreek seed yield at the first season compared to the control (Table 6). Co-inoculation significantly (P≤0.05) increased fenugreek seed yield at both seasons compared to the control.

Table.1: Effect of treatments on nodulation

Bacterial inoculation	First season			Second season		
	6 WAS	8 WAS	10 WAS	6 WAS	8 WAS	10 WAS
Control	0.10	0.01	1.56	0.94	1.53	1.17
TAL 380	0.45	3.76	2.51	2.30	2.24	3.24
BMP	0.21	1.19	0.67	2.46	2.35	1.38
TAL 380 + BMP	0.11	0.31	1.20	2.23	1.65	0.63
LSD	**0.79**	**3.37**	**1.37**	**0.51**	**0.21**	**0.35**

Table.2: Effect of treatments on nodule dry weight (mg/plant)

Bacterial inoculation	First season			Second season		
	6 WAS	8 WAS	10 WAS	6 WAS	8 WAS	10 WAS
Control	0.10	0.01	1.56	0.008	0.057	0.183
TAL 380	0.06	0.09	0.81	0.003	0.045	0.528
BMP	0.06	0.07	3.97	0.003	0.006	0.375
TAL 380 + BMP	0.04	0.01	1.20	0.002	0.007	0.150
LSD	**0.14**	**0.07**	**1.68**	**0.005**	**0.015**	**0.084**

Table.3: Effect of treatments on root dry weight (g/plant)

Bacterial inoculation	First season			Second season		
	6 WAS	8 WAS	10 WAS	6 WAS	8 WAS	10 WAS
Control	0.17	0.33	0.17	0.051	0.080	0.019
TAL 380	0.18	0.29	0.20	0.070	0.116	0.004
BMP	0.15	0.73	0.48	0.035	0.096	0.004
TAL 380 + BMP	0.07	0.20	0.27	0.040	0.130	0.003
LSD	**0.42**	**0.35**	**0.26**	**0.015**	**0.007**	**0.015**

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Table.4: Effect of treatments on shoot dry weight (g/plant)

Bacterial inoculation	First season			Second season		
	6 WAS	8 WAS	10 WAS	6 WAS	8 WAS	10 WAS
Control	1.15	2.25	2.97	0.384	0.871	0.131
TAL 380	0.54	2.53	2.22	0.658	1.299	0.253
BMP	1.83	4.13	6.17	0.361	1.524	0.139
TAL 380 + BMP	0.52	4.54	4.03	0.413	1.204	0.150
LSD	**1.18**	**2.48**	**6.12**	**0.119**	**0.109**	**0.015**

Table.5: Effect of treatments on plant height (cm), pod number and length (ml)

Bacterial inoculation	Plant height		Pod number		Pod length	
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Control	45.19	33.67	23.00	32.33	10.77	9.85
TAL 380	40.75	41.67	25.27	44.92	9.87	10.14
BMP	50.69	36.75	44.70	45.08	10.40	10.22
TAL 380+BMP	43.13	35.25	33.90	42.92	11.10	9.56
LSD	**11.06**	**6.93**	**7.58**	**5.07**	**2.22**	**1.16**

Table.6: Effect of treatments on seed/pod, 1000 seed weight (g) and yield (Kg/fedan)*

Bacterial inoculation	Seed/pod		1000 seed weight		Yield	
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Control	12.7	10.33	8.10	11.75	112.18	302.0
TAL 380	13.4	10.92	8.70	11.75	122.82	323.5
BMP	16.3	10.64	8.60	12.00	136.20	268.5
TAL 380+BMP	15.7	10.67	8.30	12.50	171.00	364.5
LSD	**5.5**	**1.30**	**1.54**	**1.69**	**10.53**	**50.07**

*Fedan = 4200 m^2

IV. DISCUSSION

The poor nodulation on the control plants indicate that there are indigenous rhizobia in the experiment soil. Inoculation with TAL380 and BMP each alone significantly increased nodule formation compared to the control. On the other hand, inoculation with TAL380 and BMP each alone or in combination significantly increased nodule dry weight at both seasons. This result is ongoing with a previous study, phosphorus solubilizing bacteria (PSB) inoculation significantly enhanced nodulation [23]. This could be due to the fact that phosphorus enhanced nodulation and nodule weight and it has direct positive effects on the indigenous rhizobia found in the rhizosphere itself [24].

Inoculation with BMP and co-inoculation significantly increased root dry weight. Moreover, inoculation with TAL380 and co-inoculation enhanced fenugreek shoot dry weight insignificantly at the first season and significantly at the second season. These results are in conformity with the findings of Bhunia *et al*. [25].

BMP increased plant height insignificantly at both seasons; inoculation with TAL380 significantly increased plant height at the second season. Similarly, El-Azouni [26] stated that phosphate solubilizing fungi inoculated soybean plants scored a significantly higher plant height.

The two bacterial inoculations each alone or in combination significantly increased number of pods at both seasons. Inoculation BMP gave the highest pod number followed by co-inoculation and TAL380, respectively. A similar result were obtained by Sharma *et al*. [23] who stated phosphorus solubilizing bacteria (PSB) inoculation significantly enhanced the pods per plant. This result can be ascribed to increasing of soluble phosphorus in the soil and this is ongoing with the findings of Ramesh *et al*. [27] who reported that increasing phosphorus rate led to increase pods per plant.

There was no effect of inoculation with bacteria on pod length. Both bacterial strains alone or in combination increased number of seeds per pod insignificantly at the two seasons. From overall mean of both seasons indicate that inoculation with BMP gave the highest number of seeds per pod, followed by co-inoculation. This result is ongoing with Kumawat *et al*. [28] who reported that seed pods were significantly higher with *Rhizobium* inoculation and with Sharma *et al*. [23] who reported that phosphorus solubilizing bacteria inoculation significantly enhanced fenugreek seeds per pod.

Inoculation with both bacterial strains alone or in combination increased 1000 seed weight insignificantly at the two seasons. In a previous study, an increment of 100 faba bean seed weight was resulted from *Rhizobium* inoculation was observed by Osman *et al*. [29]. This increment may be taken as evidence on the effect of BMP on increasing nitrogen fixation, which was reported before by Barea *et al*. [30].

From overall mean co-inoculation with TAL380+BMP gave the highest number of seeds per pod, followed by inoculation with BMP. Argaw [31] reported that dual inoculation with TAL-378 and PSB significantly increased number of seeds per pod of soybean plant compared to the other treatments.

Co-inoculation significantly increased fenugreek seed yield at both seasons. Inoculation with TAL380 and BMP each alone significantly seed yield at the first season. This result may be ascribed to the release of soluble phosphorus by BMP, Sharma *et al*. [23] and Ramesh *et al*. [27] assessed that inoculation with phosphorus solubilizing bacteria or increasing of phosphorus rate led to increase seed yield of fenugreek. Nitrogen and phosphorus are major plant nutrients and combined inoculation of *Rhizobium* sp. and PSB benefit plants more than either group of organisms alone and may have an added advantage [32].

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