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Growth, yield and chemicals constituents of fenugreek as influenced by *Rhizobium* inoculation and molybdenum foliar spray

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ABSTRACT

A field experiment was conducted at Agriculture Faculty Farm, Sebha University, Libia during the two consecutive seasons of 2009-2010 and 2010-2011 to study response of fenugreek to rhizobium inoculation (without and with inoculation) and molybdenum foliar spray as ammonium molybdate (0, 15 and 30 ppm Mo). The experiment included 6 treatments combinations in RCBD using split-plot with three replications. Results revealed that inoculation with *Rhizobium* or increasing rate of Mo significantly increased the values of most recorded parameters. In addition, the maximum fenugreek plant growth characters (plant height, number of branches and leaves per plant, plant dry weight and nodules number per plant), yield components (number of pods and seed yield per plant, seed and oil yield per hectare), seed chemical constituents (nitrogen, protein, carbohydrates, phosphorus and potassium percentage and total chlorophyll content in leaves) were achieved when plants were inoculated by *Rhizobium* and combined with foliar application with 30 ppm Mo. Thus, the better growth characters at and higher yield and its component could be obtained by application of 30 ppm molybdenum with seed inoculation by *Rhizobium*.

Key words: *Trigonella foenum graecum L.*; Fenugreek ; *Rhizobium* ; Molybdenum; Growth; Seed yield.

Introduction

Fenugreek (*Trigonella foenum graecum* L.) is an erect annual herb of the bean *Leguminosae* family. It has long been cultivated in the Mediterranean area, in India and North Africa. The seed is produced as a spice, as forage for cattle, and for medicinal purpose. Seeds also contain many substances like volatile oils, fixed oil, protein, sugar, mucilage, alkaloids and saponins, which are commercially useful as raw material for steroid hormone synthesis. The multiple uses for this plant, in food, as a spice and in medicine, as a colic flatulence in dysentery, diarrhea, as galactagolate, dyspepsia, with loss of appetite, chronic cough, enlargement of liver and spleen, gait and diabetes. Recent studies indicate that fenugreek seeds substantially contain the steroidal substance diosgenin which is used as a starting material in the synthesis of sex hormones and oral contraceptives, Shalaby and Zaki (1999) and Kamal (2007).

Inoculation of legumes is widely practiced with the objective of increasing production of the legume in question. *Rhizobium* inoculation of fenugreek has been reported to increase seed production, Poi *et al.* (1991). Fenugreek was reported to fix 40 %of its total amount of N requirement during the growing season, Desperrier *et al*. (1985). *Rhizobium* inoculation in legumes is accredited for stimulating growth and is an alternative to the expensive inorganic nitrogen fertilizers, Ndakidemi *et al*.(2007).

Molybdenum is a constituent of the nitrogenase enzyme and every bacterium which fixes nitrogen needs molybdenum during the fixation process. Molybdenum has a positive effect on yield quantity, quality and nodule forming in legume crops. Application of molybdenum into the soils has increased the contents of potassium, phosphorus and crude protein, Anonymous (2005). The transition element molybdenum (Mo) is essential for all organisms and occurs in more than 40 enzymes catalyzing diverse redox reactions, however, only four of them have been found in plants. (1) Nitrate reductase catalyses the key step in inorganic nitrogen assimilation, (2) aldehyde oxidase have been shown to catalyse the last step in the biosynthesis of the phytohormone abscisic acid, (3) xanthine dehydrogenase is involved in purine catabolism and stress reactions, and (4) sulphite oxidase is probably involved in detoxifying excess sulphite, Ralf and Hansch (2002).

The present study was undertaken to evaluate the effect of *Rhizobium* inoculation and foliar spry with molybdenum on fenugreek plants and to determine the optimum dose of those treatments for maximizing seed yield, oil yield and mucilage percentage in seeds.

Materials And Methods

The present study was conducted at Agriculture Faculty Farm, Sebha University, Libya during the two consecutive seasons of 2009-2010 and 2010-2011. The mechanical and chemical properties of the used experimental farm soil are shown in Table 1 according to Chapman and Pratt (1978).

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Seeds of fenugreek was obtained from Research Centre of Medicinal and Aromatic Plants, Dokky, Giza, Egypt and were sown on 20th and 25 th September during first and second season, respectively, to study the influence of *Rhizobium meliloti* inoculation, molybdenum foliar application and their interaction on growth,

Table 1: Physical and chemical properties of experimental farm soil

Physical properties	(%)	Chemical properties	Conc.
Sand	92.52	Total nitrogen (%)	0.018
Silt	5.48	Available phosphorus (ppm)	3.48
Clay	3.0	EC (dSm^{-1})	1.2
Soil texture	Sandy	pH	7.8
Organic matter	0.04		

yield components and chemicals constituents of fenugreek plant. The experiment included six treatments which were the combination of two *Rhizobium* inoculation, i.e. without and with, and three treatments of molybdenum as ammonium molybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, i.e. (0, 15 and 30 ppm Mo). Fenugreek seeds were inoculated *Rhizobium meliloti* before sowing. The adhesive agent used was Arabic gum 20%. The inoculated seeds were left in a shaded place for about one hour before sowing for air drying. The treatments were arranged in a split-plot design with three replicates, where *Rhizobium meliloti* inoculation were distributed in the main plots, meanwhile molybdenum concentrations were randomly arranged in the sub-plots. The experimental unit contains 5 rows each of 3 meter length and 60 cm width and the distance between plants within the row was 20 cm, where the area of each plot was 9 square meter.

After complete germination (at 20 days after sowing) plants were thinned to one plant per hill. Fenugreek plants were sprayed with the aforementioned Mo concentrations two times, at 30 and 45 days after sowing. Each experimental unit received 2 L solution using spreading agent (Supe Film at a rate of 1ml /L . The untreated plants (control) was sprayed with tap water. The normal agricultural treatments of growing fenugreek plants were practiced. Fenugreek plants were fertilized with different fertilizers at rates of 50 m^3 cattle manure, 700 kg calcium superphosphate (15.5 % P2O5), 150 kg potassium sulphate (50 % K2O) and 300 kg ammonium nitrate (33 % N) per hectare. Cattle manure, phosphorus and potassium fertilizers were applied during soil preparation. While, nitrogen fertilizer was divided into three equal doses and were added to the soil at 25, 50 and 75 days after sowing. Drip irrigation system was used in this exiperment.

Data recorded:

Plant growth parameter:

Plant height (cm), number of branches and leaves /plant, total plant dry weight (g m), and nodules number/ plant were estimated at 80 days after sowing by taken 10 plants at random from each experimental unit.

Seed yield and its component:

At harvesting stage pods number and seed yield were determined per plant and total seed yield (kg/ hectare).

Plant chemical analysis:

Sample of dry seeds were randomly taken from each treatment for chemical analysis. Oil percentage of fenugreek seeds was extracted by using petroleum ether in a soxcelt system HT apparatus according to the methods of A.O.A.C. 1984. In addition, Oil yield per plant and per hectare was calculated. Furthermore, mucilage percentage was determined only in fenugreek seeds according to the method described by Anderson (1949). Total nitrogen (%) was determined in dry seeds according to the methods described by Chapman and Pratt (1978) and was multiplied by 6.25 to calculate protein %, whereas, carbohydrate (percentage) was determined according to the method described by Dubois *et al*. (1956). Phosphorus percentage was determined according to the methods adapted by Hucker and Catroux (1980). Potassium percentage was determined by using flame photometer according to the method described by Brown and Lilleland (1964). Total chlorophyll content was determined in trigonella leaves by using a hand Spad-502 meter at 80 days after sowing .

Statistical analysis:

All collected data were statically analyzed in triplicate and the figures were then averaged. Data were assessed by analysis of variance (ANOVA) according to Snedecor and Cochran (1980). The Duncan multiple range test was used to separate means by using computer program of Statistix version

9(http://www.statistix.com/freetrial.html) . Significance was accepted at p-0.05.

Results And Discussion

1. Plant growth characters:

1.1 Effect of Rhizobium inoculation:

Data in Table 1 and Fig. 1 indicate that *Rhizobium* inoculation recorded the highest values of fenugreek number of leaves per plant, plant dry weight and nodules number per plant compared with uninoculated control in both studied seasons. *Rhizobium* inoculation did not reflect any significant effect regarding plant height and number of branches / plant in both seasons. The stimulative effect of *Rhizobium meliloti* inoculation on fenugreek growth and dry weight / plant may be due to it's ability for fixing nitrogen element from atmosphere which consider the main nutrient element for vegetative plant growth. Obtained results are in a good line with those reported by Abd-El-Ghaffar *et al*. (1994) on bean and Mehta *et al*. (2012) on fenugreek with respect to *Rhizobium* inoculation. They concluded that *Rhizobium* inoculation significantly increased plant growth and number of nodules/plant compared to uninoculated one.

Table 1: Effect of *Rhizobium* inoculation and foliar spray with molybdenum and their interaction on growth characters of fenugreek plants during the two seasons of 2009-2010 and 2010-2011

Rhizobium inoculation	Molybdenum concentration (ppm)							
	0	15	30	Mean	0	15	30	Mean
	First season				Second season			
Plant height (cm)								
Without inoculation	36.83d	40.00bcd	42.33ab	39.72 A	38.50cd	40.67bc	42.67ab	40.61A
With inoculation	38.00 cd	41.67abc	44.50a	41.39 A	35.83d	41.33bc	44.83a	40.67A
Mean	37.42 B	40.83 A	43. 42 A		37.17C	41.00B	43.75A	
Number of leaves / plant								
Without inoculation	77.17d	85.00c	90.00c	84.06 B	86.50d	91.17cd	96.17c	91.28 B
With inoculation	95.00c	105.67b	117.59a	106.06 A	95.50c	102.67b	118.83a	105.67 A
Mean	86.08C	95.33B	103.75A		91.00C	96.92B	107.50A	
Number of branches / plant								
Without inoculation	9.17 d	12.00bc	13.50abc	11.56 A	10.00e	12.00cd	13.50ab	11.83 A
With inoculation	11.50 cd	14.33ab	15.17a	13.67 A	11.00de	12.83bc	14.33a	12.72 A
Mean	10.33 B	13.17A	14.33A		10.50 C	12.42 B	13.92 A	
Plant dry weight (gm)								
Without inoculation	12.67c	15.77b	20.07a	16.17 B	13.75d	16.50c	20.30ab	16.85 B
With inoculation	15.97b	19.22a	20.70a	18.63 A	16.75c	19.42b	21.12a	19.10 A
Mean	14.32 C	17.50 B	20.38 A		15.25 C	17.96 B	20.71 A	

1.2 Effect of molybdenum foliar spray:

The effect of foliar application of molybdenum on plant growth and number of nodules / plants are presented in Table 1 and Fig 1. The data show that plant height , number of both branches and leaves /plant as well as number of nodules / plant were significantly increased due to Mo foliar application. Spraying plants with Mo at 30 ppm gave the highest values of the abovementioned plant traits compared to 15 ppm and control treatment . The increment of fenugreek dry weight might be due to the increment of vegetative growth ,i.e., plant height and number of both branches and leaves / plant.

These results are in agreement with those obtained by Bakry *et al.* (1987) , El-Mansi *et al.* (1994 and 2000) on pea and Kandil *et al.* (2013) on common bean. They reported that spraying plants with Mo at 40 ppm significantly increased vegetative growth parameter and dry weight of different plant parts of pea and common bean.

1.3. Effect of interaction between Rhizobium and molybdenum:

Obtained data in Table 1 and Fig. 1 show that a significant difference in vegetative growth characters of fenugreek plants among the interaction treatments were detected. The best interaction treatments for increasing plant height, number of branches and leaves / plant and plant dry weight as well as nodules number per plant were obtained by rhizobium inoculation combined with Mo foliar spray at 30 ppm followed by uninoculated with rhizobium combined with Mo foliar spray at 30 ppm in both seasons.

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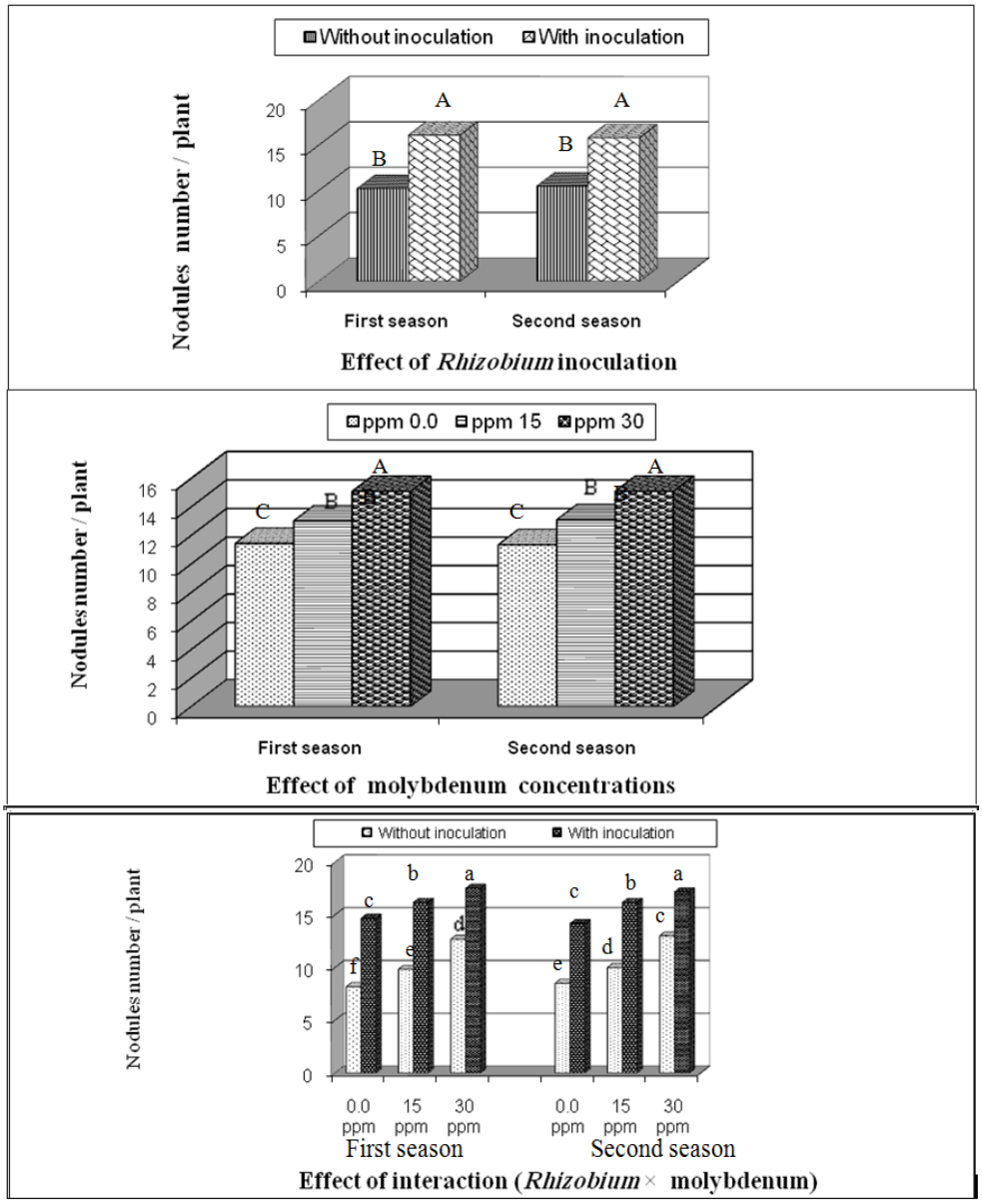

Fig. 1: Effect of *Rhizobium* inoculation and foliar spray with molybdenum and their interaction on nodules number per plant of fenugreek during the two seasons of 2009-2010 and 2010-2011.

2. Yield and its components:

2.1 Effect of Rhizobium inoculation:

Presented data in Table 2 illustrate that fenugreek plants inoculated with rhizobium gave the highest number of pods per plant, seed yield per plant and per hectare in both seasons, except seed yield / plant and hectare in the first season with did not reach 5% level of significance . Plants sprayed with Mo at 30 ppm gave the highest values of all studied parameters compared with the other used concentrations of molybdenum (0.0 or 15 ppm) in the two seasons.

The increase in yield components resulted from inoculation treatment with rhizobium is due to the stimulatory effects of biofertilizer secretion of growth regulators i.e., IAA and GA Rabie (1996). The increase in dry matter production of inoculated plants may be attributed to enhancement of nodulation, higher rate of nitrogen fixation and general improvement of root development Erum and Bano (2008). Inoculation with *Rhizobium* sp. caused a greater increase in growth and this in turn increase yield. Akhtar and Siddiqui (2009) came to similar results.

2.2 Effect of molybdenum foliar spray:

The data in Table 2 show that spraying fenugreek plants with molybdenum at 30 ppm significantly affected number of pods/ plant, seed yield/ plant and per hectare compared to control treatment . Spraying plants with Mo at 30 ppm was the most favorable treatment for increasing seed yield /plant and per hectare followed by spraying with 15 ppm and control treatment.

The increase in seed yield / plant and / hectare due to foliar application of molybdenum in fertilization process, under Mo deficiency pollen grain formed are smaller, are low in starch and have poor germination, and this in turn decrease number of seeds/pod and this directly affected seed yield (Agarwala *et al.*, 1978)

The increment in seed yield may be owe directly to the increment of vegetative growth parameter viz, plant height ,number of both branches and leaves / plant ,dry weight/ plant and the increment in enzymes activity.

These results are in agreement with those reported by Ramadan(1997) and El-Mansi *et al.*(2000) on pea plants, Bhagiya *et al.* (2005) on groundnuts and Togay *et al.* (2008) on lentil plant . They reported that spraying the above mentioned plants with Mo significantly increased number of pods/plant , pod weight , yield/ plant and total yield.

2.3. Effect of interaction between Rhizobium and molybdenum:

It is evident from the results in Tables 2 that, number of pods per plant, seed yield per plant and per hectare were significantly affected by the interaction rhizobium inoculation and Mo foliar application . The maximum seed yield and its components values (pods number per plant and seed yield per plant and per hectare) were achieved when fenugreek plants sprayed with 30 ppm in treatment where 3 gm Mo/L + inoculated with rhyzobium. The minimum values in this regard were recorded in uninoculated plant + un sprayed with Mo. These results are true in both seasons.

Table 2: Effect of *Rhizobium* inoculation and foliar spray with molybdenum and their interaction on number of pods, seed yield per plant (gm) and per hectare (kg) of fenugreek plants during the two seasons of 2009-2010 and 2010-2011

Rhizobium inoculation	Molybdenum concentration (ppm)							
	0	15	30	Mean	0	15	30	Mean
	First season				Second season			
Number of pods / plant								
Without inoculation	24.00 d	30.50 c	33.50 bc	29.33 B	27.67 e	34.33 cd	36.50 b	32.83 B
With inoculation	33.50 bc	33.83 b	41.83 a	35.78 A	32.67 d	35.00 bc	43.33 a	37.00 A
Mean	27.83 C	32.17 B	37.67 A		30.17 C	34.67 B	39.92 A	
Seed yield / plant (gm)								
Without inoculation	14.75d	16.50c	17.38ab	16.40 A	14.58d	16.37c	17.30b	16.08B
With inoculation	15.20d	16.67bc	17.82a	16.40 A	16.07d	16.65c	18.08a	16.60A
Mean	14.97C	16.64B	17.60A		14.82C	16.51B	17.69A	
Seed yield / hectare (kg)								
Without inoculation	1229.2d	1375.0c	1448.6ab	1350.9 A	1215.3d	1363.9c	1441.7b	1340.3B
With inoculation	1266.7d	1398.6bc	1484.7a	1383.3 A	1255.6d	1387.5c	1506.9a	1383.3A
Mean	1247.9C	1386.8B	1466.7A		1235.4C	1375.7B	1474.3 A	

3. Seed chemical constituents:

3.1 Effect of Rhizobium inoculation:

Data in Table 3 show that inoculation of fenugreek seeds significantly increased seed oil percentage, oil yield / plant and per hectare as well as mucilage % in seeds in the two studied seasons, except mucilage % in seeds in first season. The increment in oil yield/ha due to seed inoculation were 9.5%and 10.4% in the first season and second season respectively compared to uninoculated plants.

Regarding, N P, K, protein, carbohydrates, percentages and total chlorophyll content, data in Table 4 and Fig. 2 indicate that rhizobium inoculation increased nitrogen, carbohydrates, potassium and protein percentages of fenugreek seeds as well as chlorophyll content of fenugreek leaves, except k% in the first season. In addition, phosphorus % was not affected by rhizobium inoculation in the second season.

The increment in N% and protein % in fenugreek seeds as well as potassium , carbohydrate percentage and chlorophyll content in leaves due to rhizobium inoculation are in a good line with those reported by Arisha (1982) on pea , Abd El –Ghaffar *et al.* (1994) on bean and El-Awag (1999) on soybean.

3.2 Effect of molybdenum foliar spray:

Concerning oil percentage , oil yield/plant and per hectare as well as mucilage %in seeds of fenugreek,

Data in Table 3 indicate that the abovementioned seed oil yield components and mucilage % were increased with increasing molybdenum concentration up to the highest used concentration (30ppm) compared to 15 ppm and unsprayed plants(control). These results hold true in both seasons .

Data in Table 4 and Fig 2 show that spraying plant with molybdenum at a rate of 30 ppm increased N,P,K, protein, carbohydrate and mucilage percentages in seeds in both seasons. The highest values in this respect were obtained in plants sprayed with 30ppm molybdenum compared to control and 15ppm. Foliar spray increase of nitrogenase enzyme activity. These results may be due to the role of Mo in nitrogenase system present in all nitrogen fixing organisms. Nitrogenase enzyme consist of two different enzyme protein and Mg-ATP complex. Larger enzyme protein unit contains Fe-Mo-S complex (Srivastava and Gupto, 1996). In addition, Agarwala *et al.* (1978) found that Mo deficiency lowers the activity of catalase enzyme but stimulate the activity of peroxidase.

These results are in harmony with those reported by El-Mansi *et al.*(2000) on pea. Bhagiya *et al.* (2005) found that Mo application at a rate of 4Kg/ha increased seeds N, P and K content. In addition, Gad (2012) reported that Mo application to groundnut significantly increased seed mineral composition.

Table 3: Effect of *Rhizobium* inoculation and foliar spray with molybdenum and their interaction on oil percentage, oil yield per plant (gm) and per hectare and mucilage percentage of fenugreek plants during the two seasons of 2009-2010 and 2010-2011

Rhizobium inoculation	Molybdenum concentration (ppm)							
	0	15	30	Mean	0	15	30	Mean
	First season				Second season			
Oil percentage								
Without inoculation	10.13e	11.15c	11.85b	11.04 B	9.87e	10.95c	11.5b	10.89 B
With inoculation	10.62d	11.92b	12.85a	11.79 A	10.35d	11.90b	12.65a	11.63 A
Mean	10.37C	11.53B	12.35A		10.11C	11.42B	12.25A	
Oil yield / plant (gm)								
Without inoculation	1.49e	1.84c	2.05b	1.80 B	1.44e	1.79c	2.05b	1.76 B
With inoculation	1.61d	2.00b	2.29a	1.97 A	1.56d	1.98b	2.28a	1.94 A
Mean	1.55C	1.92B	2.17A		1.50 C	1.89 B	2.17 A	
Oil yield / hectare (kg)								
Without inoculation	124.63e	153.30c	171.52b	149.81B	120.06e	149.30c	170.74b	146.70B
With inoculation	134.55d	166.59b	190.77a	163.97A	130.04d	165.17b	190.64a	161.95A
Mean	129.59C	159.95B	181.14A		125.05A	157.24B	180.69A	
Mucilage %								
Without inoculation	31.32c	32.60bc	34.48a	32.80A	30.83e	32.70cd	34.18ab	32.57B
With inoculation	31.65c	33.77ab	34.50a	33.31A	32.00d	33.45bc	34.92a	33.46A
Mean	31.48C	33.18B	34.49A		31.42C	33.07B	34.55A	

Table 4: Effect of *Rhizobium* inoculation and foliar spray with molybdenum and their interaction on some chemical constituents of fenugreek plants during the two seasons of 2009-2010 and 2010-2011

Rhizobium inoculation	Molybdenum concentration (ppm)							
	0	15	30	Mean	0	15	30	Mean
	First season				Second season			
Nitrogen %								
Without inoculation	3.40d	3.53bc	3.62b	3.52 B	3.32b	3.28b	3.55a	3.38 B
With inoculation	3.52c	3.60bc	3.73a	3.62 A	3.35b	3.55a	3.48a	3.46 A
Mean	3.46C	3.57B	3.67A		3.33C	3.42B	3.52A	
Phosphorus %								
Without inoculation	0.525c	0.580ab	0.611a	0.572 A	0.517c	0.560b	0.601a	0.559 A
With inoculation	0553bc	0.581ab	0.510c	0.548 B	0.531bc	0.547bc	0.605a	0.561 A
Mean	0.539B	0.561AB	0.580A		0.524C	0.554B	0.603A	
Potassium %								
Without inoculation	2.57d	2.73bc	2.87ab	2.72 A	2.63b	2.73b	2.85a	2.69 B
With inoculation	2.69cd	2.85b	2.95a	2.83 A	2.48c	2.89a	2.92a	2.81 A
Mean	2.63C	2.80B	2.91A		2.56C	2.81B	2.88A	
Protein %								
Without inoculation	21.25d	22.08bc	22.60b	21.98 B	20.73b	20.52b	22.19a	21.15 B
With inoculation	21.98c	22.50bc	23.33a	22.60 A	20.94b	22.19a	21.77a	21.63 A
Mean	21.61C	22.29B	22.97A		20.83C	21.35B	21.98A	
Carbohydrates %								
Without inoculation	39.17d	39.84cd	41.20b	40.07 B	38.97d	39.90c	41.33b	40.07 B
With inoculation	40.13c	41.28b	42.17a	41.24 A	39.70c	41.52b	42.1oa	41.11 A
Mean	39.65C	40.56B	41.76A		39.33C	40.71B	41.72A	

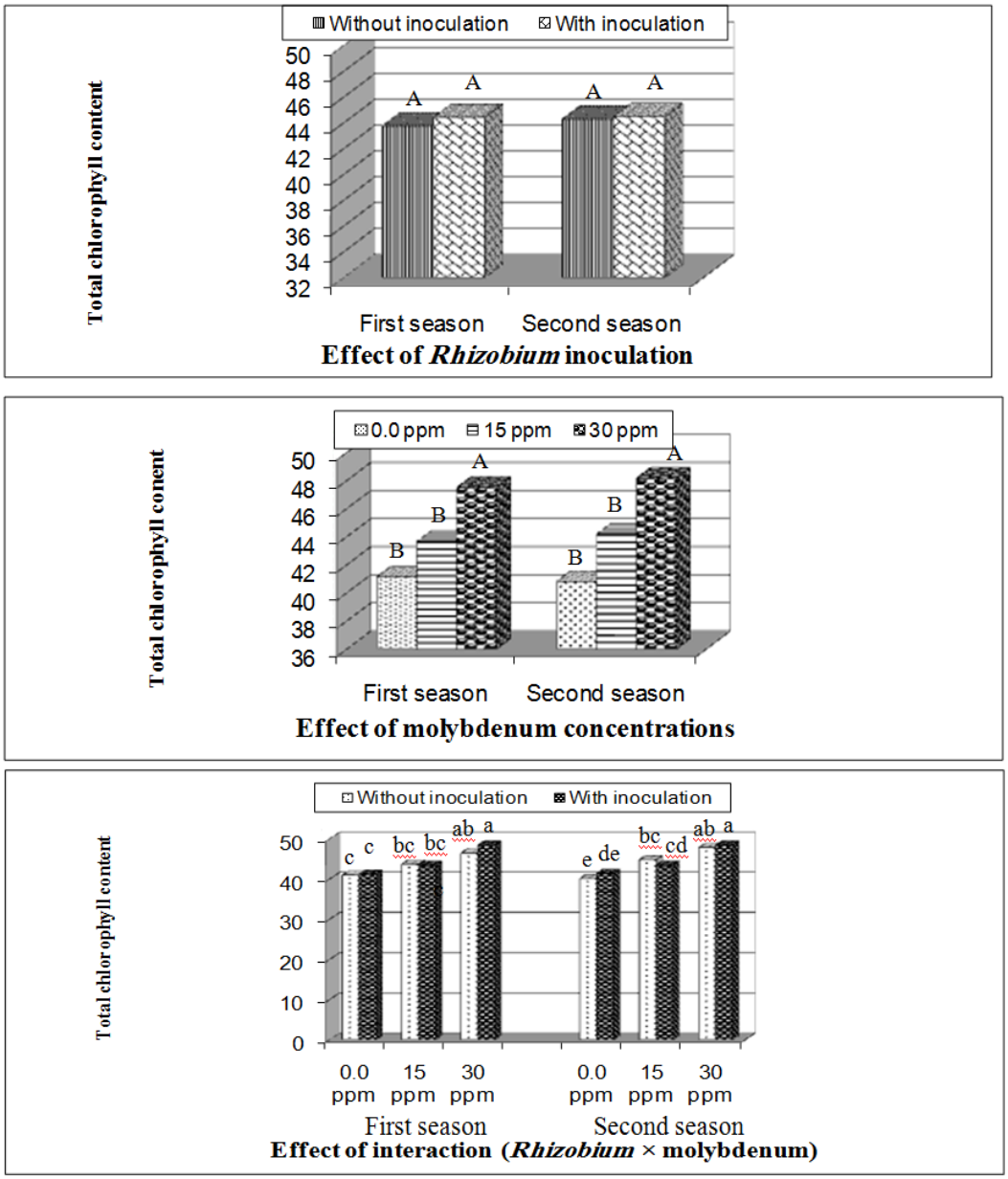

Fig. 2: Effect of *Rhizobium* inoculation and foliar spray with molybdenum and their interaction on total chlorophyll content of fenugreek leaves during the two seasons of 2009-2010 and 2010-2011

3.3 Effect of interaction between rhizobium and molybdenum:

Data in Table 3 reveal that the interaction between rhizobium inoculation and molybdenum foliar application had significant effect on oil %, oil yield per plant and per hectare as well as mucilage percentage in fenugreek seeds. It is clear from such data that, the most favorable treatment for increasing all studied seed chemical analysis (oil%, oil yield /plant, oil yield /hectare and mucilage%) was inoculation of fenugreek seeds with rhizobium x Mo foliar spray at a rate of 30ppm. The interaction of rhizobium inoculation x30ppm Mo foliar spray did not significantly differ than uninoculated plants x 30ppm Mo foliar spray.

Data in Table 4 and Fig. 2 show the affect of interaction between rhizobium and molybdenum foliar application treatments on chemical constituents of fenugreek seeds. Generally, foliar application with high level of Mo (30ppm) combined with seed inoculation with rhizobium gave the highest values of fenugreek seeds N, protein, carbohydrates, P and K percentages and leaves chlorophyll content compared with other interaction treatments.

These, results are in agreement with those obtained by Tahir *et al.* (2011), on mungbean who reported that protein content (25.55 %) was significantly higher over control when mungbean was treated with Mo (4 g/kg seed)+ inoculation with *Rhizobium*.

Conclusions:

Fenugreek has a potential to fix a substantial amount of atmospheric nitrogen. Inoculation of fenugreek with suitable strains of *Rhizobium* is recommended to improve the growth characters, seed yield and chemical constituents. Molybdenum foliar application caused an increase in all studied growth, parameters, seed yield and minerals composition as well as total chlorophyll content of fenugreek plant. Mo at 30ppm was found more efficient as compared to other Mo concentrations. Inoculation of fenugreek seed with *Rhizobium* combined with Mo foliar spray (30 ppm) was the most favorable treatment for increasing seed yield and oil yield as well as seed mucilage percentage.

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