

## Efficiency Of Biofertilization On Growth, Yield, Alkaloids Content And Chemical Constitutes of *Lupinus Termis*, L. plants

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**Abstract:** A field experiment was conducted during two successive seasons 2010/2011 and 2011/2012 to determine the influence of bio-fertilizer used were *Bacillus megatherium* var. phosphaticum (Bm) Bradyrhizobium (Rh) (used as seed inoculants) on two cultivars (Giza 1 & Giza 2) of Lupin *Lupinus termis* L. plants and their interactions. Effect of the previous treatments on vegetative growth, yield, nitrogen, phosphorus, potassium, protein, Alkaloids percentage and Alkaloids content/plant(g) of Lupin *Lupinus termis* L. plants were studied in this investigation. It is obvious that Giza 2 cultivar surpassed Giza 1 cultivar concerning plant height, plant dry weight, Pods number/plant, Pods dry weight, seed index, seed number/plant and seed yield / plant & / feddan, while Giza 1 cultivar surpassed Giza 2 cultivar concerning branch number/plant, nitrogen, phosphorus, potassium, protein percentage. There were no significant differences between the two cultivars in Alkaloids percentage and Alkaloids content/plant(g). In regard to bio-fertilizer treatments, enhanced the all parameters under study. The best results in terms of these characters were obtained when inoculating the plants with Rh + Bm + 50% mineral fertilizer followed by Rh + 50%, the combined effect between two cultivars (Giza 1 & Giza 2) and bio-fertilizer treatments on Lupin *Lupinus termis* L. plants parameters was statistically significant.

**Key words:** Bradyrhizobium, *Bacillus megatherium* var. phosphaticum, Lupin *Lupinus termis* L..

### INTRODUCTION

Lupin is considered one of the legumes with the promising future potential due to its high protein content as well as its adaptation to poor soils and dry climates. It has been used as a green manure, forage and seeds for human usage because of its high protein content. Like other seed legumes, lupin plant is able to fix atmospheric nitrogen in the soil that increase soil fertility with no additional cost or effort particularly in reclaimed soils and, therefore lupin appear to have useful effect in such areas. Inoculation of lupin seeds with rhizobium, foliar application with molybdenum and nitrogen fertilizer are among factors affecting considerably soil coverage. Ayisi *et al.* (1992) found that seed yield of inoculated plots increased by an average of 51 % in noninoculated control. Yield increase were due to changes in pod number or seed weight, depending upon the year of evaluation. Inoculation resulted in seed protein increase from 28 to 45 % greater than non-inoculated control. Also, Hob Allah *et al.* (2001) reported that inoculated plants were taller with more branches and pods / plant, more seeds / pod and higher in seed weight than the non-inoculated plants. On the other hand, Larson *et al.* (1989) working with fall-sown white lupin, found no significant difference in the yield of inoculated or non-inoculated plants by native Bradyrhizobium populations. Although lupin plant is Leguminous crop, low rate of N fertilizer may be needed for early growth and during the entire growing season when symbiotic N<sub>2</sub>-fixation by rhizobium is inhibited (Westerman *et al.*, 1981 and Eaglesham *et al.* 1983).

Bio-agriculture is one of the recent approaches to produce crops with minimal residues of chemical fertilizers in their tissues. The system is based on exploiting microorganisms with specific metabolic activities along with organic materials as a source of energy to support the proliferation in the root zone and hence it is satisfying the nutritional requirement of the growing host. The utilization of microorganisms as biofertilizers is considered today a promising alternative to mineral fertilizers. This application reduces the amount of applied mineral fertilizers and supports plant growth under less polluted conditions. From the economic point of view, such application also reduces the agricultural costs as a result of decreasing the amounts of expensive inorganic N-fertilizers and increasing the yield of inoculated crops due to providing them with an available nutrient source and growth promoting substances (Shende *et al.*, 1979; Vlassak and Reyders, 1977 & 1980; El-Demerdash *et al.* 1993 and El-Haddad *et al.*, 1993). The bifertilizers stimulate the growth, yield and chemical constituents. These results in harmony with those obtained by, Shalan *et al.* (2001) on roselle plants, Hassan *et al.* (2010) on khella plants, Hassan *et al.* (2005) on guar and fenugreek plants, Swaefy *et al.* (2007) on peppermint plant, Hassan *et al.* (2009) on black cumin plants, El-Shafie *et al.* (2010) on khella plants, Al-shaer (2011) on *Grindelia camporum* plants, stated that the application of bifertilizers on plants enhanced the growth, yield and chemical constituents.

## MATERIALS AND METHODS

This experiment was carried out in the Laboratory and Experimental Farm of Faculty of Agriculture, Al-Azhar University, Assiut, Egypt during the two successive seasons of 2010/2011 and 2011/2012 to study the effect of bio-fertilizers such as phosphate-solubilising bacteria, *Bacillus megatherium* (Bm) and nitrogen-fixing bacteria, *Bradyrhizonium* (Rh) inoculation (used as seed inoculants) on growth, yield, N,P,K & protein percentage and total Alkaloid percentage and content Lupin *Lupinus termis* L. plants.

Experimental design was laid in a split plot arrangement in completely randomized block design with three replications, Cultivars {**Giza 1**, **Giza 2**} of Lupin *Lupinus termis* L. plants considered the main plots, bio-fertilizers treatment were assigned as sub plots. Lupin seeds were sown on October 25<sup>th</sup> of the two seasons. The experimental plot was 3.6 × 3 m and contained 5 rows, 60 cm apart. The distances between the hills were 30 cm. Physical and chemical properties of the used soil are shown in Table (a). All agricultural practices were performed as usual. At the end of the experiment, the following data were recorded: plant height, number of branches / plant, plant dry weight (g) / plant, Pods number/plant, Pods dry weight (g)/plant, seed index, seed number/plant, seed yield(gm)/plant, seed yield (kg)/fed., determination of nitrogen, phosphorus, potassium & protein percentage and alkaloids (%) & content/plant (g). lupin seeds *Lupinus termis*, L. were obtained from the Department of Agronomy Lignum plants, Agriculture Research Center (A.R.C), Ministry of Agriculture, Egypt, *Bacillus megatherium* and *Bradyrhizonium* inoculation while the bio-fertilizers were brought from National Research Centre, Dokki Cairo, Egypt.

### Chemical Analysis:

Nitrogen, phosphorus and potassium percentage were determined according to A.O.A.C. (1990), The total alkaloid content of the samples was assessed using a method previously described by (Korish, 1989) which can be summarized as follows: One gram of powdered crude drought was accurately weighted and transferred to a 50 ml beaker. Five ml of ammonia solution (25 percent) were added, air-dried then 15 ml of chloroform were added and allowed to macerate for 24 hours, and filtered. The extract shaken with 45 ml of 2% sulfuric acid in a separating funnel until it tested negative for alkaloids with modified dragendorff reagent. This acid solution was adjusted to pH 10 with dilute ammonia solution (25%) and extracted with chloroform (3 times 30ml). The resulting chloroform solution, was dried over anhydrous sodium sulfate, filtered and concentrated to dryness. the residue was dissolved in 5ml chloroform, then one drop of indicator solution of gentian violet in glacial acetic acid (0.5% w/v) and 5ml. of glacial acetic acid were added. The mixture continuously stirred and titrated to a visual green end point with 0.01 N perchloric acid in glacial acetic acid. The total alkaloid content was expressed as milligrams of perivine per gram dry weight of the plant samples. Dragendorff Reagent (DR). A 0.8 g amount of bismuth nitrate was dissolved in 40 mL of distilled water and 10 mL of glacial acetic acid. The resulting solution was mixed with 20 mL of 40% potassium iodide. Calibration Cur Ve. A 18.39 mg amount of bismuth nitrate was dissolved in 5 mL of concentrated nitric acid and diluted to 100 mL with distilled water to obtain a bismuth nitrate stock solution, from which a dilution series was prepared (379.1, 284.3, 213.3, 160.0, 120.0, 90.0, 67.5, 50.6, and 38.0 µmol/L). A 5 mL amount of thiourea 3% was added to 1 mL of each solution. The absorbance value of the yellow complex formed was measured at 435 nm. The assay was performed in triplicate. The average regression equation was  $y = (6.83 \times 10^{-4} x)$ .

Ali Statistical analysis was carried out according to the method of Gomez and Gomez (1984).

**Table a:** Physico-chemical properties of the used soil.

Texture	pH	E.C. (ds /m)	CaCO <sub>3</sub> %	Available nutrients (ppm)			Water soluble Ions (meq /L) in the soil paste				
				N	P	K	Ca	Mg	CO <sub>3</sub> <sup>+</sup> HCO <sub>3</sub>	CL	SO <sub>4</sub>
Loamy	7.9	1.2	2.7	62.4	9.2	356	3.4	1.9	2.9	2.2	6.6

## RESULTS AND DISCUSSION

### The Plant Growth Parameters:

#### Plant Height:

The obtained data in Table (1) reveal that plant height of *Lupinus termis* L. had significantly affected by planting the two cultivars of Giza 1 and Giza 2 in the two experimental seasons.

It is obvious that Giza 2 cultivar surpassed Giza 1 cultivar concerning plant height ranged 7.43 and 7.77 % in the first and the second seasons, respectively.

Regarding to biofertilizer treatments , it is appear that it was significant effect on plant height of *Lupinus termis* L. for both seasons. From the obtained results, it could be noticed that all biofertilizer treatments separately or mixed with chemical fertilizer led to a significant increase in plant height as compared to untreated control in the two growing seasons.

However, it was found that inoculating *Lupinus termis* L. plants with Rh + Bm + 50% mineral fertilizer followed by Rh + 50% registered the tallest plants which increased the plant height by 24.27 and by 24.74 % over than those obtained by control in the two successive seasons respectively, as clearly shown in Table (1).

The positive effect o biofertilization on enhancing plant height was studied by many authors such as Mehasen and El-Gizawy (2004) on *Lupinus termis* L. plants, Amal G. *et al* (2010) on Chickpea plants , Hassan (2005) on guar & fenugreek plants and Hassan *et al* (2009) on black cumin plant.

Concerning the effect of the interaction , data in Table (1) pointed out that it was significant on the plant height of *Lupinus termis* L. in the two seasons. Moreover, the most effective treatments were obtained due to supplying the plants Rh + Bm + 50 % mineral fertilizer followed by Rh + 50% mineral fertilizer of Giza 2 in comparison with other combination treatments in the two consecutive seasons.

#### Branch Number:

Illustrated data in Table (1) pointed out that number of branches/plant of *Lupinus termis* L. had significantly affected by planting the two cultivars of Giza 1 and Giza 2 in the two seasons. However, it is obvious that Giza 1 cultivar surpassed Giza 2 cultivar concerning branch number ranged 11.68 % in the second season.

In regard to biofertilization treatment, the data reveal that all of them significantly augmented branch number of *Lupinus termis* L. in both seasons. However, the maximum values of branch number was observed when adding Rh + Bm + 50% mineral fertilizer which increased it by 60.57 % and by 67.77 % over control plants in the first and the second seasons, respectively .

The stimulating effect of biofertilization in increasing number of branches/plant was reported by Hassan (2005) on guar and fenugreek plants , Hassan *et al* (2009) on black cumin plants ,Hassan *et al.* (2010) on khella and plants Al-Shaer (2011) on *Grindelia camporum* plants .

The interaction between some cultivars and bio-fertilizer treatments on number of branches / plant had significant effect in the two successive seasons. It is obvious that Giza 2 cultivar plus inoculating the plants with Rh + Bm + 50% mineral fertilizer gave the highest values of branch number in comparison with those obtained by other combination treatments in the two experimental seasons, as clearly shown in Table (1).

#### Plant Dry Weight:

Data presented in Table (1) show that the plant dry weight of *Lupinus termis* L. had significantly affected by planting the two cultivars of Giza 1 and Giza 2 in the two seasons.

It is obvious that Giza 2 cultivar heaviest from Giza 1 cultivar ranged 35.04 and 35.17 % in the first and the second seasons, respectively.

Plant dry weight of *Lupinus termis* L. plants was significantly affected by inoculation treatments with Rh, B.m and Rh + B.m and with or without 50 % mineral fertilizer in the two experimental seasons. From the obtained results, it is noticed that all of them led to a significant increase in plant dry weight in comparison with the check treatment in the first and the second seasons. Inoculating the plants with Rh + B.m + 50 % mineral fertilizer gave better plant dry weight which increased it by 76.31 and by 64.44 % over the control plants in both seasons, respectively, as clearly shown in Table (1).

**Table 1:** Effect of biofertilizers (Rh &Bm) on growth of two cultivar of *Lupinus termis* L plant

Biofertilizers(B)	First season			Second season		
	Cultivar(A)			Cultivar(A)		
	Plant height(cm)					
	Giza 1	Giza 2	Mean	Giza 1	Giza 2	Mean
Cont.	99.7	105.5	102.6	101.3	107.3	104.3
Rh	110.5	121.7	116.1	112.8	124.0	118.4
Bm	109.5	120.8	115.1	109.6	122.9	116.3
Rh+Bm	115.5	127.1	121.3	115.6	127.4	121.5

Rh+50%	121.6	130.4	126.0	124.0	131.5	127.8
Bm+50%	119.2	124.4	121.8	121.4	127.2	124.3
Rh+Bm+50%	124.5	130.5	127.5	126.3	133.9	130.1
Mean	114.4	122.9		115.9	124.9	
L.S.D.0.05	A: 1.61 B:0.87 AB: 1.24			A: 1.90 B:1.27 AB: 1.79		
	Branch number/plant					
Cont.	14.6	13.6	14.0	14.53	13.27	13.9
Rh	18.6	18.07	18.33	19.80	18.50	19.15
Bm	14.6	13.53	14.07	17.30	17.10	17.2
Rh+Bm	20.03	19.47	19.75	21.40	19.87	20.63
Rh+50%	22.5	21.33	21.92	24.13	21.20	22.67
Bm+50%	20.87	17.53	19.2	20.63	17.03	18.83
Rh+Bm+50%	24.33	20.63	22.48	25.43	21.27	23.32
Mean	19.36	17.74		20.46	18.32	
L.S.D.0.05	A: ns B:1.04 AB: 1.47			A: 2.12 B:0.62 AB: 0.87		
	Plant dry weight(gm)					
Cont.	12.03	15.00	13.51	13.74	17.15	15.44
Rh	16.61	23.51	20.06	18.42	23.68	21.05
Bm	15.01	21.36	18.18	14.76	22.45	18.61
Rh+Bm	17.35	23.96	20.66	19.31	26.55	22.93
Rh+50%	19.44	25.45	22.45	20.36	26.49	23.43
Bm+50%	18.26	24.23	21.25	19.18	26.23	22.71
Rh+Bm+50%	20.36	27.27	23.82	21.43	29.34	25.39
Mean	17.01	22.97		18.17	24.56	
L.S.D.0.05	A: 1.88 B:1.04 AB: 1.47			A: 1.43 B:0.86 AB: 1.22		

The stimulating effect of biofertilization in increasing plant dry weight was reported by Hassan (2005) on guar and fenugreek plants, Hassan *et al* (2009) on black cumin plants, Hassan *et al.* (2010) on khella plants and Al-Shaer (2011) on *Grindelia camporum* plants.

The interaction between some cultivars and bio-fertilizer treatments on plant dry weight had significant effect in the two successive seasons. It is obvious that Giza 2 cultivar plus inoculating the plants with Rh + Bm + 50% mineral fertilizer gave the highest values of plant dry weight in comparison with those obtained by other combination treatments in the two experimental seasons, as clearly shown in Table (1).

#### **Pods Number/Plant:**

The obtained data in Table (2) reveal that Pods number/plant of *Lupinus termis* L. had significantly affected by planting the two cultivars of Giza 1 and Giza 2 in the two experimental seasons.

It is obvious that Giza 2 cultivar surpassed Giza 1 cultivar concerning Pods number/plant ranged 9.91 and 8.62 % in the first and the second seasons, respectively.

Regarding to biofertilizer treatments, it is appear that it was significant effect on Pods number/plant of *Lupinus termis* L. for both seasons. From the obtained results, it could be noticed that all biofertilizer treatments separately or mixed with chemical fertilizer led to a significant increase in Pods number/plant as compared to untreated control in the two growing seasons.

However, it was found that inoculating *Lupinus termis* L. plants with Rh + Bm + 50% mineral fertilizer followed by Rh + 50% given the maximum Pods number/plant by 53.13& 66.68 and by 53.38 & 75.94 % over than those obtained by control in the two successive seasons respectively, as clearly shown in Table (2).

Concerning the effect of the interaction, data in Table (2) pointed out that it was significant on the Pods number/plant of *Lupinus termis* L. in the first season. Moreover, the most effective treatments were obtained due to supplying the plants Rh + Bm + 50 % mineral fertilizer of Giza 2 in comparison with other combination treatments.

#### **Pods Dry Weight(Gm)/Plant:**

Data presented in Table (2) show that Pods dry weight (gm)/plant of *Lupinus termis* had significantly affected by planting the two cultivars of Giza 1 and Giza 2 in the two experimental seasons.

Moreover, it was found that the heaviest weight of Pods dry Giza 2 cultivar than Giza 1 cultivar *Lupinus termis* L. plants by 14.84 and by 15.36 % in the two growing seasons, respectively.

Pods dry weight of *Lupinus termis* L. plants was significantly affected by inoculation treatments with Rh, B.m and Rh + B.m and with or without 50 % mineral fertilizer in the two experimental seasons. From the obtained results, it is noticed that all of them led to a significant increase in Pods dry weight in comparison with the check treatment in the first and the second seasons. Inoculating the plants with Rh + Bm + 50 % mineral fertilizer gave better Pods dry weight which increased it by 47.92 and by 47.95 % over the control plants in both seasons, respectively, as clearly shown in Table (2).

The interaction between some cultivars of *Lupinus termis* L. plants and bio-fertilizer treatments on Pods dry weight (gm)/plant had significant effect in the first season. It is obvious that Giza 2 cultivar plus inoculating

the plants with Rh + Bm + 50% mineral fertilizer gave the highest values of Pods dry weight (gm)/plant in comparison with those obtained by other combination treatments in the two experimental seasons, as clearly shown in Table (2)

**Seed index:**

Illustrated data in Table (2) pointed out that seed index of *Lupinus termis* had significantly affected by planting the two cultivars of Giza 1 and Giza 2 in the two seasons. However, it is obvious that Giza 2 cultivar surpassed Giza 1 cultivar concerning seed index ranged 6.74 and 4.84 % in the two growing seasons, respectively.

In regard to biofertilization treatment, the data reveal that all of them significantly augmented seed index of *Lupinus termis* L. in both seasons. However, the maximum values of seed index was observed when adding Rh + Bm + 50% mineral fertilizer which increased it by 34.47 % and by 36.24 % over control plants in the first and the second seasons, respectively .

The stimulating effect of biofertilization in increasing 100 seed weight was reported by EL Hadi & Elsheikh (1999), Amal G. *et al* (2010) on Chickpea (*Cicer arietinum* L.) plants

The interaction between two cultivars ( Giza 1 &Giza 2) and bio-fertilizers treatments on seed index was insignificant in both seasons.

**Table 2:** Effect of biofertilizers (Rh &Bm) on Pods number & dry weight and seed index of two cultivar of *Lupinus termis* L plant

Biofertilizers(B)	First season			Second season		
	Cultivar(A)			Cultivar(A)		
	Pods number/plant					
	Giza 1	Giza 2	Mean	Giza 1	Giza 2	Mean
Cont.	16.30	20.43	18.37	18.37	21.53	19.95
Rh	23.33	25.47	24.40	24.50	25.70	25.10
Bm	21.60	23.30	22.45	22.30	24.27	23.28
Rh+Bm	25.80	27.23	26.52	28.63	30.50	29.57
Rh+50%	27.83	28.43	28.13	29.80	31.40	30.60
Bm+50%	24.20	29.17	26.68	26.87	30.43	28.65
Rh+Bm+50%	29.73	31.50	30.62	33.87	36.33	35.10
Mean	24.11	26.50		26.33	28.60	
L.S.D.0.05	A: 1.32 B:1.26 AB: 1.79			A: 0.33 B:2.15 AB: ns		
	Pods dry weight(gm)/plant					
Cont.	17.12	19.43	18.28	17.34	21.66	19.50
Rh	21.83	23.86	22.85	21.98	23.82	22.90
Bm	19.00	21.42	20.21	19.92	22.46	21.19
Rh+Bm	22.52	26.11	24.32	23.30	26.84	25.07
Rh+50%	23.71	27.67	25.69	24.60	28.35	26.48
Bm+50%	21.30	26.18	23.74	23.22	28.32	25.77
Rh+Bm+50%	25.43	28.64	27.04	27.28	30.42	28.85
Mean	21.56	24.76		22.52	25.98	
L.S.D.0.05	A:1.58 B:0.83 AB: 1.18			A: 0.45 B:1.32 AB: ns		
	Seed index					
Cont.	24.75	26.12	25.44	25.43	27.05	26.24
Rh	31.20	33.41	32.31	32.52	34.17	33.35
Bm	29.67	32.03	30.85	30.92	33.08	32.00
Rh+Bm	32.72	34.42	33.57	34.25	35.58	34.92
Rh+50%	32.32	34.41	33.36	34.08	35.25	34.67
Bm+50%	31.35	33.88	32.62	33.08	34.75	33.92
Rh+Bm+50%	33.11	35.32	34.21	35.05	36.42	35.75
Mean	30.73	32.80		32.20	33.76	
L.S.D.0.05	A: 0.62 B:0.45 AB: ns			A: 0.27 B:0.38 AB: ns		

**The Yield Parameters:**

**Seed Number/Plant:**

Data recorded in Table (3) reveal that Seed number/plant of *Lupinus termis* L. had significantly affected by planting the two cultivars of Giza 1 and Giza 2 in the second season only.

It is obvious that Giza 2 cultivar surpassed Giza 1 cultivar concerning Seed number/plant ranged 15.12 % in the second seasons.

Concerning bio-fertilizer treatments, data in Table (3) show that the influence of them on Seed number/plant was significant in both seasons. From the obtained results, it is noticed that the highest number of seed were detected due to inoculating *Lupinus termis* L. plants with Rh + Bm + 50% mineral fertilizer reached 75.88 and 77.85 % over the check control for the two seasons, respectively

The stimulating effect of biofertilization in increasing Seed number/plant was reported by Hassan (2005) on guar and fenugreek plants , Hassan *et al* (2009) on black cumin plants.

The interacting effect between two cultivars (Giza 1 & Giza 2) and bio-fertilizer treatments on Seed number/plant was significant in the second season only. The most effective treatments were obtained from treating Giza 2 cultivar with Rh + Bm + 50 % mineral fertilizer comparison with other combination treatments, as clearly shown in Table (3).

#### Seed Yield Per Plant And Per Feddan:

Data presented in Table (3) reveal that Seed yield per plant and per feddan of *Lupinus termis* L. had significantly affected by planting the two cultivars of Giza 1 and Giza 2 in the two experimental seasons.

It is obvious that Giza 2 cultivar surpassed Giza 1 cultivar Seed yield per plant and per feddan ranged 13.62 and 14.10 % and per feddan with 13.59 and 14.10 % in both seasons, respectively. The differences among soybean cultivars in growth parameters may be due to the differences in number of nodules formed in roots of tested cultivars, This finding by Tawfic *et al* (1991) on chickpea plants also differences in partition and migration o photosynthetic between cultivars by Ahmed *et al* (1997) on *Vicia faba* L. plants.

Seed yield of *Lupinus termis* L. per plant and per feddan was significantly affected by inoculated the plants with Rh, B.m and Rh + B.m and with or without 50 % mineral fertilizer treatments in the two experimental seasons.

**Table 3:** Effect of biofertilizers (Rh &Bm) on yield of two cultivar of *Lupinus termis* L. plant

Biofertilizers(B)	First season			Second season		
	Cultivar(A)			Cultivar(A)		
	Seed number/plant					
	Giza 1	Giza 2	Mean	Giza 1	Giza 2	Mean
Cont.	15.33	16.93	16.13	16.47	17.57	17.02
Rh	21.40	25.03	23.22	25.53	27.73	26.63
Bm	21.23	24.17	22.70	23.83	25.23	24.53
Rh+Bm	24.27	27.07	25.67	24.00	30.00	27.00
Rh+50%	26.30	27.53	26.92	26.07	30.73	28.40
Bm+50%	27.27	26.67	26.97	24.83	30.07	27.45
Rh+Bm+50%	27.03	29.70	28.37	27.80	32.73	30.27
Mean	23.26	25.30		24.08	27.72	
L.S.D.0.05	A: ns B:1.33 AB: ns			A: 2.05 B:1.29 AB:1.83		
	Seed yield(gm)/plant					
Cont.	16.67	18.67	17.67	18.00	21.17	19.58
Rh	24.50	26.33	25.42	27.00	29.33	28.17
Bm	20.50	24.83	22.67	22.33	27.67	25.00
Rh+Bm	26.50	29.00	27.75	28.83	31.00	29.92
Rh+50%	26.50	30.00	28.25	28.33	32.50	30.42
Bm+50%	23.00	28.33	25.67	25.00	30.50	27.75
Rh+Bm+50%	27.83	30.83	29.33	30.17	32.83	31.50
Mean	23.64	26.86		25.67	29.29	
L.S.D.0.05	A:1.58 B:0.63 AB: 0.89			A: 0.45 B:0.77 AB: 1.08		
	Seed yield (kg)/fed.					
Cont.	432.1	483.9	458.0	466.7	548.7	507.7
Rh	635.2	682.7	658.9	700.0	760.5	730.2
Bm	531.5	643.8	587.6	579.0	717.3	648.1
Rh+Bm	687.0	751.6	719.3	747.5	803.7	775.6
Rh+50%	687.0	777.8	732.4	734.5	842.6	788.6
Bm+50%	596.3	734.5	665.4	648.1	790.7	719.4
Rh+Bm+50%	721.6	799.4	760.5	782.1	851.2	816.6
Mean	612.9	696.2		665.4	759.2	
L.S.D.0.05	A: 40.95 B:16.39 AB: 23.18			A: 11.57 B:19.85 AB:28.07		

From the obtained results, it could be noticed that the highest seed yield / plant and / feddan were observed when treating *Lupinus termis* L. plants with Rh + Bm + 50 % mineral fertilizer which increased it by 65.99 and 60.88 % over the control in the two seasons, respectively. These aforementioned treatments recorded 760.5 and 816.6 Kg / feddan seed in comparison with untreated ones (458.0 and 507.7) Kg / feddan seed in the first and the second seasons, respectively, as clearly illustrated in Table (3).

The efficiency of BM and VAM on improving seed yield was detected by EL Hadi & Elsheikh (1999), Amal G. *et al* (2010) on Chickpea (*Cicer arietinum* L.) plants and Awad *et al* (2010) on Faba bean (*Vicia faba* L.) plants.

The interaction between two cultivars (Giza 1 & Giza 2) and bio-fertilizer treatments on seed yield / plant and / feddan had significant effect for the two successive seasons. From the obtained data it is clear that inoculating the plants with Rh + Bm + 50 % mineral fertilizer and Giza 2 cultivar gave the maximum seed yield in the first and the second seasons, respectively. These above treatments yielded 799.4 and 851.2 kg / feddan

seed in the two seasons, respectively, while the check treatment amounted (483.9 and 548.7 ) kg / feddan seed in both seasons, respectively.

This increases might be related to the positive effect of microorganisms increasing the root surface area permit of soil volume, water – use efficiency and photosynthetic activity, which directly effects the physiological presses and utilization of carbohydrates also might be microorganisms effects on growth characteristics and chemical constitutes this finding by Abd Elaziz (2007)

#### Chemical Constituents:

##### N,P, K and Protein Percentage:

Data recorded in Table (4) pointed out that nitrogen, phosphorus , potassium and protein percentage of *Lupinus termis* L. had significantly affected by planting the two cultivars of Giza 1 and Giza 2 in the two experimental seasons.

**Table 4:** Effect of biofertilizers (Rh &Bm) on chemical constituents of two cultivar of *Lupinus termis* L plant

Biofertilizers(B)	First season			Second season		
	Cultivar(A)			Cultivar(A)		
	N %					
	Giza 1	Giza 2	Mean	Giza 1	Giza 2	Mean
Cont.	4.307	4.427	4.367	4.360	4.120	4.240
Rh	5.317	4.423	4.870	5.403	4.613	5.008
Bm	4.963	4.643	4.803	5.107	4.450	4.778
Rh+Bm	5.463	5.080	5.272	5.600	5.207	5.403
Rh+50%	5.423	5.023	5.223	5.553	5.100	5.327
Bm+50%	5.137	4.943	5.040	5.260	5.033	5.147
Rh+Bm+50%	5.527	5.213	5.370	5.630	5.273	5.452
Mean	5.016	4.822		5.273	4.828	
L.S.D.0.05	A: 0.27 B:0.28 AB: 0.39			A: 0.07 B:0.08 AB:0.12		
	P %					
Cont.	0.203	0.183	0.193	0.227	0.207	0.217
Rh	0.283	0.220	0.252	0.310	0.233	0.272
Bm	0.333	0.253	0.293	0.360	0.267	0.313
Rh+Bm	0.360	0.277	0.318	0.373	0.290	0.332
Rh+50%	0.327	0.237	0.300	0.337	0.280	0.308
Bm+50%	0.330	0.280	0.305	0.347	0.293	0.320
Rh+Bm+50%	0.367	0.293	0.330	0.390	0.317	0.353
Mean	0.315	0.254		0.335	0.270	
L.S.D.0.05	A:0.01 B:0.01 AB: 0.01			A:0.01 B:0.01 AB:0.02		
	K %					
Cont.	0.723	0.653	0.688	0.750	0.663	0.707
Rh	0.790	0.727	0.758	0.823	0.760	0.792
Bm	0.750	0.697	0.723	0.773	0.720	0.747
Rh+Bm	0.810	0.750	0.780	0.833	0.777	0.805
Rh+50%	0.797	0.743	0.770	0.823	0.770	0.797
Bm+50%	0.767	0.743	0.755	0.797	0.747	0.772
Rh+Bm+50%	0.807	0.760	0.783	0.860	0.787	0.823
Mean	0.778	0.725		0.809	0.746	
L.S.D.0.05	A: 0.01 B:0.01 AB: 0.02			A: 0.02 B:0.01 AB: ns		
	Protein %					
Cont.	26.92	27.67	27.29	27.25	25.75	26.50
Rh	33.23	27.65	30.44	33.77	28.83	31.30
Bm	31.02	29.02	30.02	31.92	27.87	29.87
Rh+Bm	34.15	31.75	32.95	35.00	32.54	33.77
Rh+50%	33.90	31.40	32.65	34.71	31.88	33.29
Bm+50%	32.11	30.90	31.50	32.88	31.46	32.17
Rh+Bm+50%	34.54	32.59	33.57	35.19	32.96	34.08
Mean	32.27	30.14		32.96	30.18	
L.S.D.0.05	A: 1.71 B:1.74 AB: 2.46			A: 0.46 B:0.51 AB: 0.72		

It is clear that Giza 1 cultivar surpassed Giza 2 cultivar concerning nitrogen, phosphorus , potassium and protein percentage ranged 4.02, 24.02, 7.31&7.07 % and 9.22, 24.07, 8.45 & 9.21 % in both seasons, respectively.

Regarding to biofertilizer treatments, it is appear that it was significant effect on nitrogen, phosphorus , potassium and protein percentage of *Lupinus termis* for both seasons. From the obtained results, it could be noticed that all biofertilizer treatments separately or mixed with chemical fertilizer led to a significant increase in nitrogen, phosphorus , potassium and protin percentage as compared to untreated control in the two growing seasons.

However, it was found that inoculating *Lupinus termis* L. plants with Rh + Bm + 50% mineral fertilizer registered which increased the nitrogen, phosphorus, potassium and protein percentage by 22.97, 70.98, 13.80 & 23.01 % and by 28.58, 62.67, 16.41 & 28.60 % followed by Rh + Bm by 20.72, 64.77, 13.37 & 20.74 and 27.36, 52.99, 13.86 & 27.43 over than those obtained by control in the two successive seasons respectively, as clearly shown in Table (4).

The positive effect of biofertilization on enhancing nitrogen, phosphorus, potassium and protein percentage was studied by many authors such as Awad *et al* (2010) on Faba bean (*Vicia faba* L.) plants, Hassan *et al* (2009) on black cumin plant, Swaefy Hend *et al.* (2007) on peppermint plant, Al-Shaer (2011) on *Grindelia camporum* plants and Harun & Hussein (2003) on *Lupinus termis* L plants.

Concerning the effect of the interaction, data in Table (4) pointed out that it was significant on the nitrogen, phosphorus, potassium and protein percentage of *Lupinus termis* in the two seasons exception effect between cultivars and bio-fertilizers on potassium insignificant in the second season only. Moreover, the most effective treatments were obtained due to supplying the plants Rh + Bm + 50 % mineral fertilizer followed by Rh + Bm mineral fertilizer of Giza 1 in comparison with other combination treatments in the two consecutive seasons.

#### **Alkaloids percentage:**

The obtained data in Table (5) reveal that Alkaloids percentage of *Lupinus termis* L. had significantly affected by planting the two cultivars of Giza 1 and Giza 2 in the two experimental seasons.

It is obvious that Giza 1 cultivar surpassed Giza 2 cultivar concerning Alkaloids percentage ranged 12.74 and 11.83 % in the first and the second seasons, respectively.

Regarding to biofertilizer treatments, it is appear that it was significant effect on Alkaloids percentage of *Lupinus termis* L. for both seasons. From the obtained results, it could be noticed that all biofertilizer treatments separately or mixed with chemical fertilizer led to a significant increase in Alkaloids percentage as compared to untreated control in the two growing seasons.

However, it was found that inoculating *Lupinus termis* L. plants with Rh + Bm + 50% mineral fertilizer followed by Rh + 50% registered the increased the Alkaloids percentage by 72.23 & 64.17 and by 69.70 & 61.21 % over than those obtained by control in the two successive seasons respectively, as clearly shown in Table (5).

The positive effect of biofertilization on enhancing Alkaloids percentage was studied by many authors such as Hassan (2012) on periwinkle (*Catharanthus roseus* L.) plants.

Concerning the effect of the interaction, data in Table (5) pointed out that it was significant on the Alkaloids percentage of *Lupinus termis* L. in the two seasons. Moreover, the most effective treatments were obtained due to supplying the plants Rh + Bm + 50 % mineral fertilizer followed by Rh + 50 % mineral fertilizer of Giza 1 in comparison with other combination treatments in the two consecutive seasons.

**Table 5:** Effect of biofertilizers (Rh & Bm) on Alkaloids % and content of two cultivar of *Lupinus termis* L plant

Biofertilizers(B)	First season			Second season		
	Cultivar(A)			Cultivar(A)		
	Alkaloids (%)					
	Giza 1	Giza 2	Mean	Giza 1	Giza 2	Mean
Cont.	1.363	1.143	1.253	1.397	1.243	1.320
Rh	2.120	1.860	1.990	2.183	1.910	2.047
Bm	1.673	1.560	1.617	1.720	1.597	1.658
Rh+Bm	2.173	1.920	2.047	2.210	1.987	2.098
Rh+50%	2.163	1.950	2.057	2.213	2.043	2.128
Bm+50%	1.933	1.663	1.798	1.997	1.713	1.855
Rh+Bm+50%	2.267	2.050	2.158	2.370	2.110	2.240
Mean	1.956	1.735		2.013	1.800	
L.S.D.0.05	A: 0.07 B:0.04 AB: 0.05			A: 0.05 B:0.03 AB:0.05		
	Alkaloids content/plant(g)					
Cont.	0.229	0.214	0.221	0.249	0.263	0.256
Rh	0.524	0.491	0.507	0.591	0.562	0.577
Bm	0.434	0.388	0.366	0.386	0.443	0.414
Rh+Bm	0.581	0.555	0.568	0.639	0.617	0.628
Rh+50%	0.579	0.587	0.583	0.629	0.666	0.647
Bm+50%	0.449	0.472	0.460	0.501	0.524	0.512
Rh+Bm+50%	0.633	0.633	0.633	0.715	0.695	0.705
Mean	0.477	0.477		0.530	0.539	
L.S.D.0.05	A: ns B:0.02 AB: 0.03			A: ns B:0.02 AB:0.03		

#### **Alkaloids Content/plant(g):**

The obtained data in Table (5) reveal that Alkaloids content/plant(g) of *Lupinus termis* had significantly affected by planting the two cultivars of Giza 1 and Giza 2 in the two experimental seasons.

Regarding to biofertilizer treatments, it is appear that it was significant effect on Alkaloids content/plant(g) of *Lupinus termis* L. for both seasons. From the obtained results, it could be noticed that all biofertilizer treatments separately or mixed with chemical fertilizer led to a significant increase in Alkaloids percentage as compared to untreated control in the two growing seasons.

However, it was found that inoculating *Lupinus termis* L. plants with Rh + Bm + 50% mineral fertilizer followed by Rh + 50 % registered the increased the Alkaloids content/plant(g) by 186.42 & 163.80 and by 175.39 & 152.73 % over than those obtained by control in the two successive seasons respectively, as clearly shown in Table (5).

The positive effect of biofertilization on enhancing Alkaloids content/plant(g) was studied by many authors such as Hassan (2012) on periwinkle (*Catharanthus roseus* L.) plants.

Concerning the effect of the interaction, data in Table (5) pointed out that it was significant on the Alkaloids content/plant(g) of *Lupinus termis* L. in the two seasons. Moreover, the most effective treatments were obtained due to supplying the plants Rh + Bm + 50 % mineral fertilizer followed by Rh + 50% mineral fertilizer of Giza 1 and Giza 2 in comparison with other combination treatments in the two consecutive seasons.

This increases might be related to the positive effect of microorganisms increasing the root surface area per unit of soil volume, water – use efficiency and photosynthetic activity, which directly effects the physiological processes and utilization of carbohydrates also might be microorganisms effects on growth characteristics and chemical constituents this finding by Abd Elaziz (2007)

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