

# Enhancement the Growth and Phenolic Content of Faba Bean (*Vicia faba* L.) by Applying Some Biofertilizer Agents

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## Abstract

Some microorganisms, i.e., *Rhizobium leguminosarum* var. *faba*, *Aphanocapsa albida* and *Laurencia obtusa*, were applied in pots to enhance the growth of faba bean (*Vicia faba* L.) plants. The individual and combined microorganisms applications significantly increased the plant height and number of leaves at the early growth stages. Ninety days after planting, all the treatments significantly enhanced the plant growth parameters. *Laurencia obtusa* + *Aphanocapsa albida* increased the whole plant length, dry weight, total phenols content and nitrogen content by 46.3%, 96.5%, 315% and 86% respectively. However while the combination between the three microorganisms increased whole plant fresh weight by 56.0% in comparison with control.

**Keywords:** Faba bean, Cyanobacteria, Rhizobium, Marine algae, Phenolic content

## Introduction:

Faba (*Vicia faba* L., ) bean is an annual legume which also known botanically as also known as the broad bean, fava bean, faba bean, field bean, bell bean, or tic bean, is a species of bean (Fabaceae) native to north Africa, southwest and south Asia, and extensively cultivated elsewhere. Faba bean roots need to be inoculated with the appropriate strains of rhizobia (*Rhizobium leguminosarum*), which will infect the plants root and stimulates root nodule development. This occurs when the faba beans are being grown in the field for the first time or where they have not been grown for long time.

Biofertilizers are the micro-organisms and their products which are utilized to increase soil fertility. The N<sub>2</sub> - fixing micro-organisms are *Anabaena*, *Nostoc*, *Rhizobia*, *Tolypothrix* etc. Biofertilizers are products containing living cells of different types of microorganisms which when applied to seeds, plant surface or soil, colonize the rhizosphere or the interior of the plant and promotes growth by converting nutritionally important elements (nitrogen and phosphorus) from unavailable to available form through biological process such as nitrogen fixation and solubilization of rock phosphate (Rokhzadi *et al.*, 2008). El-Yazid *et al.* (2007) found that beneficial microorganisms in biofertilizers accelerate and improve plant growth and protect plants from pests and diseases.

The property of symbiotically fixing nitrogen within nodules of vascular plants is found in two major groups of bacteria one of them, rhizobia that associate essentially with leguminous, (Sprent, 2007). Another important group of nitrogen fixing bacteria is that of the cyanobacteria, found in association with a large variety of higher and lower plants, fungi and algae. (Meeks and Elhai, 2002). Franche *et al.* (2009) reported that leguminous plants can obtain their nitrogen by association with rhizobia via differentiation on their respective host plants of a specialized organ, the root nodules. Bhattacharyya and Pati, (2000) found that *Rhizobium* sp., produced high amount (107µg/ml) of IAA in culture from tryptophan supplemented Yeast Extract Mannitol medium. The production was maximum when the bacteria reached its stationary phase of growth.

Other symbiotic associations involve hetero-cystous cyanobacteria, while increasing numbers of nitrogen-fixing species have been identified as colonizing the root surface and, in some cases, the root interior of a variety of cereal crops and pasture grasses. Kevin Vessey, (2003) found that rhizobacteria when used as biofertilizer can influence root growth, morphology and other beneficial plant microbe symbioses. Cyanobacteria are one of the major components of the nitrogen fixing biomass in paddy fields. Due to the important characteristic of nitrogen fixation, cyanobacteria have a unique potential to contribute to enhance productivity in a variety of agricultural and ecological situations. It plays an important role to build up soil fertility consequently increasing in the yield, ( Sahu *et al.*, 2012 and Song *et al.*, 2005).

Thirumaran *et al.* (2009) stated that recent researches proved that seaweed fertilizers are preferred not only due to their nitrogen, phosphorus and potash content but also because of the presence of trace elements and metabolite similar to plant growth regulators. Recently, seaweed extracts as liquid fertilizers (SLF) has come in the market for the simple reason that

they contain many growth promoting hormones like auxin, gibberellin, trace elements, vitamins, amino acids and micronutrients. Strik *et al.* (2004) reported that the seaweeds extracts are effective fertilizers in many crops.

The objectives of this study are to observe the effect of individual and combined *Rhizobium leguminosarum* var. *faba*, *Aphanocapsa albida* and *Laurencia obtusa* applications on the growth, total phenols content and nitrogen content of faba bean (*Vicia faba* L.).

## Materials and Methods

### Rhizobium preparation

Commercial formula known as "Okkadeen" biofertilizer contained *Rhizobium leguminosarum* var. *faba* was obtained from Legume Crops Dept., Field Crops Research Institute, A R C, Giza, Egypt.

One gm of the Okkadeen biofertilizer was suspended in 100 ml sterilized distilled water and shaken well. Serial dilutions were made by taking a loop of each of them to 100 ml sterilized distilled water. A loop from  $10^{-9}$  dilution was transferred to 100 ml Yeast Extract Manitol "YEM" medium and incubated in a water bath shaker at  $25^{\circ}\text{C}\pm 1$  for 72 hrs. Seeds were immersed in sugar solution; as an adhesive material; prepared by dissolving 20 gm of sugar in 100 ml water. Treated seeds were then mixed thoroughly with the "Okkadeen" biofertilizer and left for 30 min. in a shadow place for drying before cultivation.

### Algae collection and preparation

*Laurencia obtusa* was collected in May, 2011 from shallow water beside the shore of Red Sea at Safaga. After collection, algae were washed with fresh sea water to remove the epiphytes, sand and other extraneous matter then they were dried in shadow open air and completing the drying process in the oven at  $60^{\circ}\text{C}$  for 5 hours. Then, dried algae were ground to fine powder by mechanic grinder. The algae were applied as a soil treatment at the rate of 3 gm powdered algae/Kg soil seven days before planting and watered twice daily.

The blue green algae, *Aphanocapsa albida* were isolated from soil. Isolation and purification of algae were done according to the method described by Rippka, (1988). Algae were isolated after repeated light migrations on solid BG11, medium, (Zarrouk, 1966 and Stainer *et al.*, 1971). They were grown in Erlenmeyer flasks (500 ml) in axenic conditions. The cultures were incubated in the room temperature of approx  $25\pm 2^{\circ}\text{C}$  and a light intensity 2500 lux. provided by cool, white ,fluorescent tubes under continuous illumination for 15 days. Two hundred ml. from the culture was added to the pots after planting.

### Plant material

Seeds of Faba bean (*Vicia faba* L.) cv. Sakha1 were surface sterilized with ethanol 70% and washed by sterile distilled water, then dried in shadow open air.

The seeds were planted in 30 cm diameter earthen pots containing mixture of 1:1 autoclaved peat and sand soil. Every pot contained 2 seeds. They watered every week.

## Growth measurements

Plant height and number of leaves were recorded at different growth stages. At the end of the experiment; plant height, plant fresh weight, plant dry weight, N - content and Phenol content were recorded.

## The treatments

1-Control, 2- *Rhizobium leguminosarum* var, *faba*, 3- *Aphanocapsa albida* , 4- *Laurencia obtusa* + *Aphanocapsa albida*, 5- *Rhizobium leguminosarum* var, *faba* + *Aphanocapsa albida* ,6- *Rhizobium leguminosarum* var, *faba* + *Laurencia obtusa* + *Aphanocapsa albida*.

## Chemical analysis

### 1-Determination of total phenolics content (TPC)

The determination of total phenolics content (TPC) was estimated spectrophotometrically by using Folin-Ciocalteu reagent as described by (Singleton and Rossi, 1965). The absorbance was measured at 750 nm and compared to gallic acid calibration curve. The TPC was calculated by comparing the absorbance with the gallic acid calibration curve according to the formula:

TPC (mg/g) = C x V / g where;

C = concentration of the gallic acids equivalent from standard curve (mg/mL)

V = volume of the extract used (ml)

g = weight of extract (g)

The contents were expressed as gallic acids (mg GAE/g dry wt).

### 2- Determination of N-content

The determination of total nitrogen was carried out with Micro-Kjeldahl method. (Anonymous 1990). Oh point five g of dried and finely ground shoot and root sample was taken in a Kjeldahl flask. Three g of digestion mixture ( $H_2SO_4 + K_2SO_4$ ) in the ratio of 1:9 was added and followed by 20 ml of  $H_2SO_4$ . The sample was boiled in digestion apparatus for 1.5-2 hrs. until the contents became clear. The digested material was cooled and diluted up to 250 ml in a volumetric flask by adding distilled water. An aliquot 10 ml of it was transferred to the micro Kjeldahl distillation apparatus. It was mixed with 10 ml of 40 % NaOH and distilled in a receiver containing 10 ml of 2 % boric acid solution with methyl red as indicator. The contents of the distillate were titrated against standard sulfuric acid (N/10  $H_2SO_4$ ) to light pink color end point. From the volume of acid used, percentage of nitrogen was calculated based on ammonia liberated.

## Statistical analysis

The responses of the treatments were compared by analysis of variance (ANOVA) (Sokal and Rohlf, 1995). Significant differences between the means of parameters were determined by

using Duncan's multiple range tests ( $P \leq 0.05$ ). All analysis was carried out with Spss software.

## Results

*Rhizobium leguminosarum* var. *faba*, *Laurencia obtusa* and *Aphanocapsa albida* and their combinations were used to study their effect as biofertilizers agents to enhance the growth of Faba bean (*Vicia faba* L.). *In Vivo*. Studies; it could be noticed that the application of *Rhizobium leguminosarum* var. *faba* + *Aphanocapsa albida* caused significant increase in plant height after 15 days (Fig., 1) while *Laurencia obtusa* + *Aphanocapsa albida* gave the best results after 30, 45, 60 and 75 days in comparison with control.

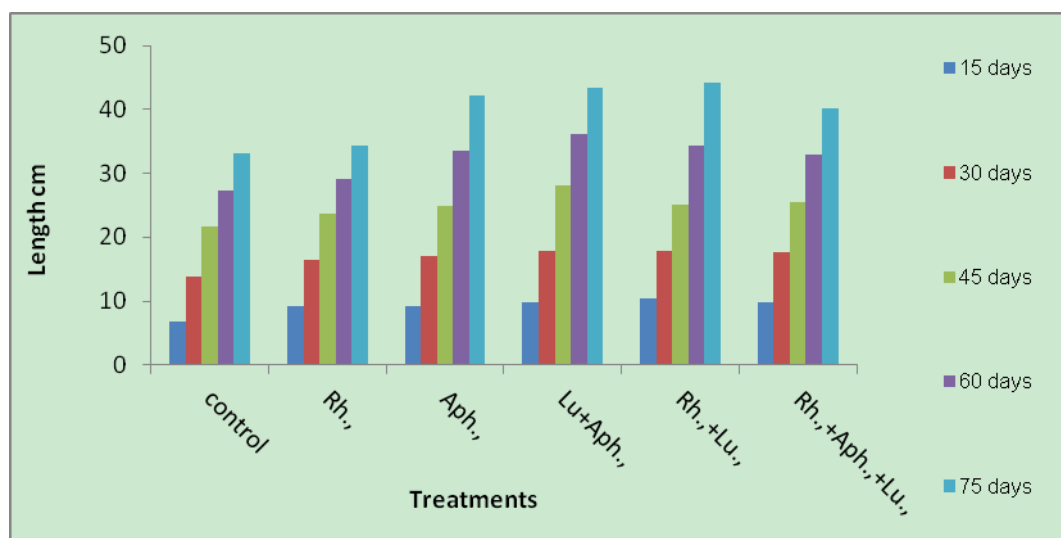


Figure 1. Effect of some biofertilizer agents on plant height of faba bean (*Vicia faba* L.)

Results in table 1 clear that number of leaves after 15, 30, 45, 60 and 75 days was increased in response to variable treatments in comparison with control. Application of *Rhizobium leguminosarum* var. *faba* + *Laurencia obtusa* + *Aphanocapsa albida* gave the same result of control. Generally it could be noticed that *Laurencia obtusa* + *Aphanocapsa albida* and *Rhizobium leguminosarum* var, *faba* + *Laurencia obtusa* were the best treatment for plant growth at the early stages from planting.

Table 1. Effect of some biofertilizers agents on leaves number of faba bean (*Vicia faba* L.)

Treatments	Leaves number				
	15 days	30 days	45 days	60 days	75 days
Control	6.6 a	8.30 a	10.0a	11.2a	12.5 a
Rh.,	8.0 b	9.5 ab	11.0 ab	12.1 ab	13.2 ab
Aph.,	8.0b	9.2 ab	10.5 ab	12.1ab	13.7 b
Lu.,+Aph.,	8.0 b	9.6 b	11.2 ab	12.5 ab	13.8 b
Rh.,+ Lu.,	7.9 b	10.4 b	13.0 b	13.5 b	14.0b
Rh.,+ Aph., +Lu.,	8.0b	9.6b	11.2 ab	11.8 a	12.5 a
L S D <sub>0.05</sub>	.043	.014	.136	.024	.003

Rh.,: *Rhizobium*    Aph.,: *Aphanocapsa*    Lu.,: *Laurencia*

The criteria based on plant length, fresh and dry weight, N- content and total phenol content were calculated after 90 days from planting (flowering stage). Average length of shoot, root and whole plant are shown in table 2 which clear that all the treatments increased the whole plant length (21.6-46.3%). Application of *Laurencia obtusa* + *Aphanocapsa albida* caused the best increase of shoot, root and whole plant length, (46.2) in comparison with control.

 Table 2. Effect of some biofertilizers agents on length (cm) of faba bean (*Vicia faba* L.)

Treatments	length (cm)			
	Shoot	Root	Total plant	Increasing%
Control	37.2a	22.4 a	59.6 a	-
Rh.,	40.0 ab	32.5 b	72.5 ab	21.6
Aph.,	45.5 d	29.4 ab	74.9 ab	25.6
Lu.,+Aph.,	46.4 d	40.8 bc	87.2 b	46.3
Rh.,+ Lu.,	41.7bc	34.5 bc	76.1ab	27.6
Rh.,+ Aph., +Lu.,	45.1 cd	35.0 c	80.1 b	34.3
L S D <sub>0.05</sub>	.000	.000	.035	

Rh.,: *Rhizobium*    Aph.,: *Aphanocapsa*    Lu.,: *Laurencia*

Table 3 illustrates that all the treatments gave significant increase in whole plant fresh weight ranged between (11.3-56.0%). The treatment of *Rhizobium leguminosarum* var, *faba* + *Laurencia obtusa* + *Aphanocapsa albida* gave the best result of whole plant fresh weight which reached 56.0% compared with control. Also all the treatments caused significant

increase in shoot, root and whole plant dry weight (57.2-96.5%). *Laurencia obtusa* + *Aphanocapsa albida* gave the best result and caused 96.5% inc

Table 3. Effect of some biofertilizers agents on fresh and dry weight (gm) of faba bean (*Vicia faba* L.)

Treatments	Fresh weight (gm)				Dry weight (gm)			
	Shoot	Root	Whole plant	Increasing%	Shoot	Root	Whole plant	Increasing%
Control	9.4a	4.2 a	13.7a	-	1.62a	1.2a	2.8a	-
Rh.,	10.4ab	4.8ab	15.2a	11.3	2.1ab	2.9c	5.1bc	81.1
Aph.,	14.9c	6.2bc	21.1 bc	54.8	2.2abc	2.2b	4.4b	57.2
Lu.,+Aph.,	12.8 bc	8.2 d	20.3bc	48.5	2.9c	2.6bc	5.6c	96.5
Rh.,+ Lu.,	13.7 c	4.9ab	18.7b	36.7	2.1ab	3.0 c	5.2bc	82.5
Rh.,+ Aph., +Lu.,	14.0 bc	7.3 cd	21.3	56.0	2.4bc	2.7bc	5.1 bc	780.7
L S D <sub>0.05</sub>	.004***	.000***	.000***		.007***	.000***	.000***	

**Rh.,:** *Rhizobium*    **Aph.,:** *Aphanocapsa*    **Lu.,:** *Laurencia*

rease of whole plant dry weight comparing with the control.

Data in Figure 2 showed that application of *Laurencia obtusa* + *Aphanocapsa albida* caused the highest increase of total phenols content in Faba bean plant followed by *Rhizobium leguminosarum* var, *faba* treatment which reached 315% and 86% consequently.

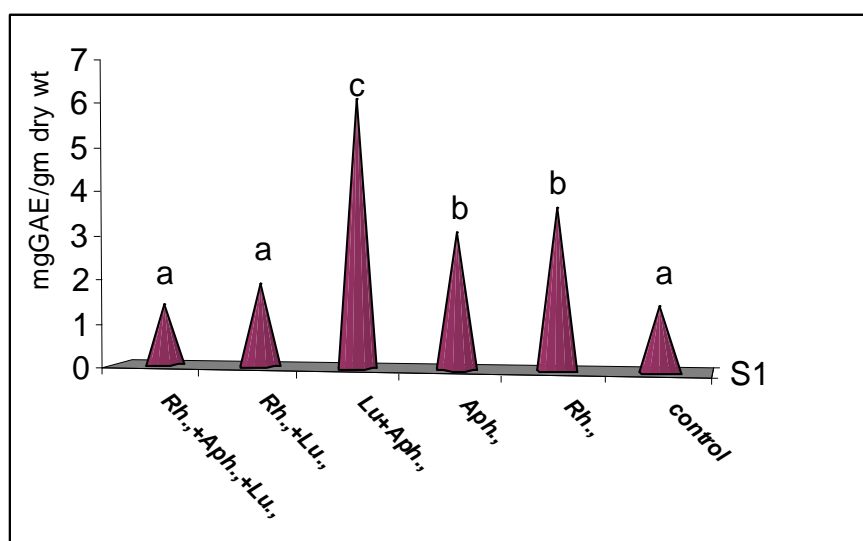


Figure 2. Effect of some biofertilizers agents on total phenole of faba bean (*Vicia faba* L.)

Figure 3 illustrates the mean percentage in nitrogen content in shoot root and whole plant of faba bean. The obtained results indicate that all the treatments caused increase in both of shoot, root and whole plant nitrogen content. *Laurencia obtusa* + *Aphanocapsa albida* caused the best result which reached 85.71 % followed by *Aphanocapsa albida* which caused 80.95 % increasing in comparison with control.

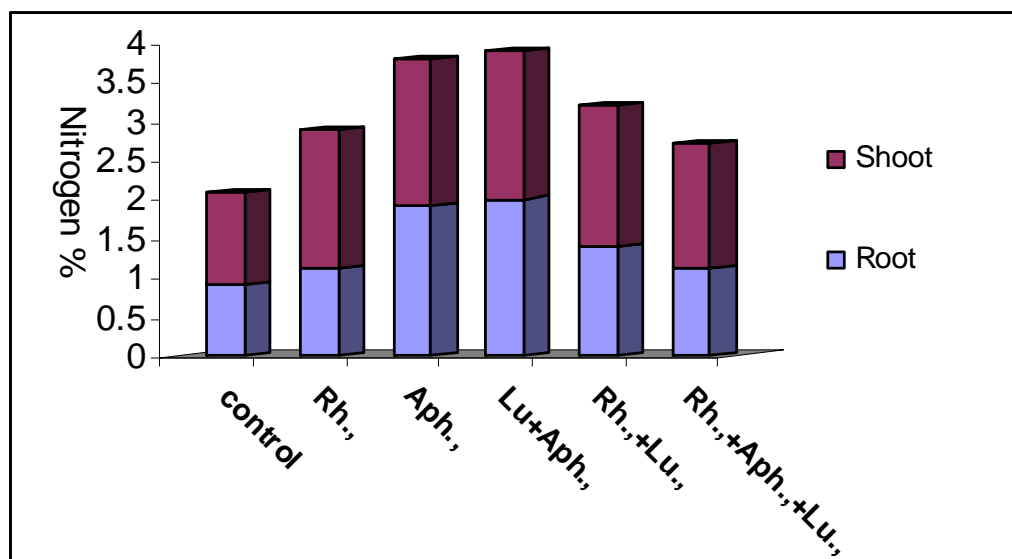


Figure 3. Effect of some biofertilizers agents on total nitrogen content of faba bean (*Vicia faba* L.)

## Discussion

Obtained results indicate that *Rhizobium leguminosarum* var., *faba*, *Laurencia obtusa* and *Aphanocapsa albida* and their combinations have significant potential as biofertilizers agents to enhance the growth of Faba bean (*Vicia faba* L.). These results are in agreement with Rokhzadi et al. (2008), El-Yazied et al. (2007), Sahu et al. (2012), Song et al. (2005), Sergeeva et al. (2002), Prasanna et al. (2002), Strik et al. (2004) and Kevin Vessey, (2003). At the early growth stages the all used treatments increased plant height and number of leaves. These results are also obtained by Ravishankar, (2002), Guirgis et al. (2007), El-Barody et al. (2007) and Haroun and Hussein, (2003). After 90 days, at the end of the experiment, application of the studied microorganisms caused noticeable increase in plant length, fresh weight and dry weight of faba bean plant. These results are in agreement with those obtained by Faheed and Abd-El Fattah, (2008). Nitrogen content of treated plants were increased and these results are also noticed by Haroun and Hussein, (2003), Adam, (1999), Lozano et al. (1999), Subramaniyan and Malliga, (2011) and Saleh et al. (2008).

## Conclusion

All biofertilizer treatments increased the growth parameters at all the tested plant stages while *Laurencia obtusa* + *Aphanocapsa albida* was the best tested treatment to enhance the plant growth parameters. Thus using combination of marine algae i.e; *Laurencia obtusa* and



cyanobacteria i.e; *Aphanocapsa albida* as biofertilizers agents improved the growth of faba bean (*Vicia faba* L.) better than *Rhizobium leguminosarum* var. *faba* alone.

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