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EFFECT OF BIO-AND MINERAL NITROGEN FERTILIZERS ON GROWTH AND YIELD OF SNAP BEAN GROWN IN SUBSTRATE CULTURE.

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Abstract

This study was conducted under unheated plastic house condition at Arid Land services and Research Center (ALARC), Faculty of agriculture - Ain Shams University, Egypt, during two successive autumn seasons 2007/2008 and 2008/2009 ability of use to investigate the *Rhizobium* inoculation as bio-fertilizer to reduce the use of mineral nitrogen fertilizers in the substrate culture and its effect on the growth and yield of Snap bean (*phaseolus vulgaris L.*) cv. Goya

Four different mineral nitrogen rates (25%, 50%, 75% and 100% of 200 ppm) with *Rhizobium* inoculation compared to 100% mineral N without inoculation also two different types of substrate systems (containers and pots) were in this investigation. The experimental design was split plot with three replicates.

Vegetative growth, plant height, chlorophyll reading (spad), total leaves area, early and total yield, chemical analysis of pods, total soluble solids (T.S.S.%), total protein, total nitrogen content, number of nodules and nitrogenase activity were measured.

Data showed that container system had a significant effect on vegetative growth parameters (plant height, chlorophyll reading (spad), and total leave area), and fruit weight (early and total fruit weight). Chemical properties of pods, number of nodules and nitrogenase activity compared to pots system.

Control treatment (100% N without inoculation) and (100% N with inoculation) recorded the highest vegetative growth, early and total yield, T.S.S.(%), pod protein and total nitrogen content followed by 50 % N with *Rhizobium* inoculations. Moreover, data showed that 50% N gave the highest number of nodules and nitrogenase activity.

Data showed that there were no significant among controls, 100%, 75% and 50% N combined with container and 100% N combined with pots followed by control combined with pots. Increase nitrogen rates led to increase the values of plant height, chlorophyll reading (spad), early yield and total yield.

Keywords: Snap bean (*phaseolus vulgaris L.*), substrate system, *Rhizobium* inoculation, mineral nitrogen, container system, pots system and Nitrogen fixing activity.

Introduction

Snap Bean (*phaseolus vulgaris L.*) is an important legume crop worldwide and Egypt for local consumption as well as for exportation. However, it's sensitive to environmental conditions (climate, salinity, irrigation and fertilization and etc..,). It can grow by assimilation on mineral nitrogen or molecular N fixation. Common bean is usually considered a poor nitrogen-fixing (N_2-fixing) legume however, its promising potential to fix nitrogen has been shown in several studied **(Peix *et al.*, 2001; Garcia *et al.*, 2004; Remans *et al.*, 2008).**

Biological nitrogen fixation (BNF) is the microbiological process, which converts atmosheric dinitrogen gas into a plant usable form. All organisms, which reduce nitrogen to ammonia, do so with the aid of the enzyme complex “nitrogenase”. These bacteria can fix nitrogen through their free living in the soil or through the association with a plant host. The microorganism,s ability to fix nitogen and the amount of fixation that takes place is strongly influenced by soil conditions. Factors such as moisture, temperature, oxygen supply, diseases and insects can also affect the degree of nitrogen fixation **(Subba-Rao, 1976).**

The importance of symbiotic nitrogen fixation is has been overemphasized due to the fact that the protein requirement worldwide has been on a rise and serious environmental problems have emered during the production and use of fertilisers based on minerals and nitrogen. Given the requirement for a 20.000 Kcal energy in order to produce 1 Kg of fertiliser containing nitrogen and the solar energy was used for binding of nitrogen biologically (microorganism-plant photosynthesis-N fixation), the importance of biological nitrogen fixation could much more clearly be understood. It is estimated that the ammount of biologically obtained nitrogen is approximately 175×10^6 tons. This amount is expected to be the dinding nitrogen symbiotically**(Partier, 1978).** Inoculation with *Rhizobium* strain ISRA 355 and nitrogen fertilizer application (0,20 or 80 kg N/ha) were significant on shoot and root dry weights mainly. However, pod yields and nodules dry weight were significantly higher in inoculated and nitrogen (20 kg N/ha) treated plots than in uninoculated: +77 % and +300% respectively **(Adama *et al.,* 2008).**

Therefore, an alternative way to meet the increasing demands of N fertilizer, to achieve maximum production and to avoid the soil problems is to use substrate culture technique to produce the important crops. The growing medium used in substrate culture must have good nutrient and water holding characteristics, and provide good aeration to the root system. Light weight is another important consideration so that filled bags can be easily handled. Growing media should be free of pathogens and substances that are toxic to plant. The principal materials that meet these requirements are few**. (Wilson, 1983).** To achieve maximum production, greenhouse vegetables in general need a well-aerated soil with a high water holding capacity, rich in nutrients and free of pathogens Although the soil is the natural rooting medium for plants, the fact that the soil in greenhouse is under cover gives rise to a lot of problems, besides the difficulty and cost of eradicating the soil borne pests and diseases of protected vegetables has led to the development of soilless substrates **(Adams, 1990; Gul and Sevgican, 1992).** Fumigation with soil sterilants such as methyl bromide has been a common practice. With the loss of this soil sterilant in 2005, growers have begun to shift to Soilless culture **(Cantliffe *et al.*, 2003)**.

The aim of this study was to investigate the ability of use *Rhizobium* inoculation to reduce the use of chemical nitrogen fertilizers in the substrate culture and its effect on the growth and yield of Snap bean (*phaseolus vulgaris L.*) cv. Goya

Material and Methods

The experiment was carried out at the experimental farm of the Arid Land Services and Research center (ALARC), Faculty of Agriculture - Ain Shams

University, Egypt, under unheated plastic house (9 x 30 x 3.5 m), for two autumn seasons of 2007/2008 and 2008/2009 using closed substrates culture.

Seeds of snap bean (*phaseolus vulgaris L.*) cv. Goya (round type) were used in this experiment. The seeds were sown on the last week of October in both seasons in foam trays (x cell) contain peat moss mixed with vermiculite (1:1 v/v) and transplanted in substrate systems on November after two weeks of sowing in both seasons. Three seedling of snap bean were planting together in the same hole in each substrate system. The final plant spacing was 40 cm in the row, 40 cm between the rows, and 70 cm in between the double rows.

Two substrate systems have been used in this experiment; pot system and container system. The pot system consisted of 10 L volume/3 plants (25 cm diameter x 30 cm length) and the container system consisted of 120 L volume (35 cm width x 20 cm depth x 3 m length) to perform 6 plants holes (20 L/3 plants). One substrate was tested in this experiment namely peat moss and sand with compost (47.5%: 47.5%: 5% – v/v respectively). Sand was primarily washed with diluted nitric acid to get rid from the undesirable salts, then with running tap water to wash nitric acid compounds from the sand. After sand was getting dry, it mixed with peat moss and compost. The used substrate technique contain: Blake polyethylene 200 micron thickness, 75 cm wide and 3.4 m length to cover bricks bins 35 cm width x 20 cm depth x 3 m length to perform the main gully which fill by substrate directly to presented the container system or arrange pots inside. A plastic tank 60 L. (one per each gully) and submersible pump was used (one per each tank) to pump the nutrient solution in the polyethylene pipe (16 mm) via dripper 2 litters per hour. The nutrient solution returns back to the solution tank by gravity with slope (1.5%). The fertigation was programmed via digital timer to work 3-4 times/day and the duration of irrigation time depended upon the season.

One isolate of *Rhizobium Leguminosaum bv. phaseoli* from Unit of Biofertilizers, Dept. Microbiology, Faculty of Agriculture, Ain Shams University, The isolates were completely purified and maintained on Yeast Extract Mannitol medium YEM in liquid form, as well as the imported strains of rhizobia **(Vincent, 1970).** The inoculation applied beside the plants after 14 days from Trans planting.

Four different concentrations of mineral nitrogen in the nutrient solutions were tested in this experiment: 100 % N, 75 % N, 50 % N and 25 % N (200 ppm, 150 ppm, 100 ppm and 50 ppm respectively) as presents in Table (1). The composition of nutrient solution described by **(El-Behiary, 1994)** was used as the 100% N treatment without *Rhizobium* inoculation. The other nitrogen treatments were prepared by using different nitrogen quantities.

The adjustment of EC m. mhos. Cm^{-1} was according to the plant consumption by adding water and concentrated nutrient solution wherever the level of nutrient solution in the catchment's tank arrives to 25% of tank volume. Digital EC meter was used to adjust the EC to the demand level. The pH of the nutrient solution was ranged 6.0 – 6.5. Data in Table (1) presents the composition of the control nutrient solution.

Treatments:

The experiment included ten treatments as follows:

1-Container control (100 % mineral N without *Rhizobium* inoculation)
2-Container + inoculation *Rhizobium* + 100 % mineral N
3-Container + inoculation *Rhizobium* + 75 % mineral N
4-Container + inoculation *Rhizobium* + 50 % mineral N
5-Container + inoculation *Rhizobium* + 25 % mineral N
6-Pots control (100 % mineral N without *Rhizobium* inoculation)

7-Pots + inoculation *Rhizobium* + 100 % mineral N
8-Pots + inoculation *Rhizobium* + 75 % mineral N
9-Pots + inoculation *Rhizobium* + 50 % mineral N
10-Pots + inoculation *Rhizobium* + 25 % mineral N

Measurements

Plant height, chlorophyll reading (spad) were determined by (Minolta chlorophyll meter Spad-501), Total leaves area/plant (cm^2) were measured after 75 days from transplanting. Early and Total yield determined in the end of both growing seasons by accumulated the harvest weight.

Total soluble solids (T.S.S. %) were determined in green pods by refractometer **(A.O.A.C., 1990)**. Total protein was determined as gm/100 gm dry weight using Micro- Kjeldahle method according to **(Piper 1947)**. Total nitrogen content assayed according to (**Pregle 1945)**, using the micro- Kjeldahl apparatus.

After 60 days of planting, plant samples were carefully uprooted and collected to determine the number of nodules per plant. Nitrogen fixing activity of nodulated roots was estimated by the acetylene reduction assay (ARA) according to (**Hardy *et al*., 1973).**

The experimental design was Split plot with three replicates where substrate systems were distributed in the main plots while, N concentrations in the nutrient solution allocated in sub plots. Analysis of data was done by computer, using SAS program for statistical analysis. The differences among means were tested for significance at 5 % level according to **(Snedecor and Cochran 1980).**

All other agriculture practices of snap bean cultivation were in accordance with standard recommendations for commercial growers by the Egyptian Ministry of Agriculture.

Table 1. The different concentration of nitrogen levels in nutrient solutions.

Nutrient	**Concentrations of nutrient solution**			
	100 % N	**75 % N**	**50 % N**	**25 % N**
Macro elements (ppm)				
N	200	150	100	50
P	45	45	45	45
K	350	350	350	350
Ca	200	200	200	200
Mg	50	50	50	50
Micro elements (ppm)				
Fe	3.0	3.0	3.0	3.0
Zn	0.25	0.25	0.25	0.25
Mn	0.75	0.75	0.75	0.75
Cu	0.15	0.15	0.15	0.15
B	0.25	0.25	0.25	0.25

Results and Discussion

The effect of nodulation and substrate culture:

Vegetative growth

Plant height (cm/plant)

Data in Table (2) illustrated the effect of different nitrogen rates and substrate systems on plant height (cm/plant) of snap bean plants grown under condition of N_2-fixing. Regarding the effect of substrate system, data showed that the container system recorded higher significant plant height than pot system.

There is no effect for nitrogen rate between the control, 100 % N and 50 % N treatment. Regarding the effect of N_2-fixing, data showed that using 25 % reduced plant height comparing with 75 % significantly, along the two experimental seasons. Referring the effect of interaction, combined with container system, control, 50% and 100% N recorded the highest plant height comparing with the other interaction without significant differences among them. On the other hand, the lowest plant height was obtained by plants grown in pot system combined with 25% N.

Chlorophyll reading (spad)

The effect of different nitrogen rates and substrate systems on chlorophyll reading (spad) of snap bean plants grown under condition of N_2-fixing as shown in Table (3). Data indicated that using pots system significantly reduced chlorophyll reading (spad) comparing with using container system significantly in the first season but in the second season, there were non significant between substrate systems.

Regarding the effect of nitrogen rates, data illustrated that the control treatment increased chlorophyll reading (SPAD) followed by using 100% and 50% N treatment. While using 25% N treatment gave the lowest chlorophyll reading (spad). Similar trend were observed in the second season.

The interaction effect, data showed that the control combined with container or pot systems gave the highest values of chlorophyll content during the both seasons. The differences between the treatments 100% and 50% N combined with container were insignificant of chlorophyll reading (spad) in the both seasons. The lowest chlorophyll was obtained by 25% N.

Total leave area (cm^2 / plant)

The results presented in Table (4) the effect of different nitrogen rates and substrate systems on total leaf area (cm^2/plant) of snap bean plants grown under condition of N_2-fixing during the two studied seasons.

The data showed that the container system recorded the higher leaves area compared to the pots system. And the difference between them was significant.

Concerning the effect of nitrogen rates, data illustrated that control treatment gave the highest total leave area of snap bean plants in comparison with the other treatments combinations during the two working seasons. The lowest records were preceded by 25% N.

On the other hand, the interaction effect results showed that the control combined with container system gave the highest total leave area in the both seasons followed by 100% N combined with container. The lowest values were recorded by 25% N combined with pots system in both seasons.

Similar results were obtained according to **(Yadegari *et al.*, 2008).** The treatment of *Rhizobium* inoculations with 50 % N gave similar effect such as 100 % N and control due to N_2 fixing *Rhizobium.* The effect of *Rhizobium* inoculations was equal to 50 % N of nitrogen addition.

While the container system offered better condition for plant growth compared to the pots. Potentially, container volume (size) can important role in controlling plant growth. Larger volumes generally favor increased plant growth rates; however, most of these studies were done with transplants grown in restricted cell sizes **(Cantliffe, 1998).** Thus container size becomes important when considering costs for media as well as cost and durability of the container.

Table 2. Effect of different mineral nitrogen rates with Rhizobium inoculation compared to 100% mineral N without inoculation and substrate systems on plant height (cm/plant) of snap beans cv. Goya during (2007/2008) and (2008/2009) autumn seasons.

Treatments	Control	25% N	50% N	75% N	100% N	Mean
			First season			
Container	250 a	192.5 c	245 a	216 b	240 a	**288.8 A**
Pots	175 d	146 f	160 e	161 e	175 d	**163.6 B**
Mean	**212.5 A**	**169.5 C**	**202.5 A**	**189 B**	**207.5 A**	
			Second season			
Container	265 a	204 c	259 a	229 b	245 a	**242.5 A**
Pots	185 d	155 f	169 e	171 e	185 d	**173.4 B**
Mean	**225.2 A**	**179.7 C**	**214.5 A**	**200.5 B**	**219 A**	

Table 3. Effect of different mineral nitrogen rates with Rhizobium inoculation compared to 100% mineral N without inoculation and substrate systems on chlorophyll reading (spad) of snap beans cv. Goya during (2007/2008) and (2008/2009) autumn seasons.

Treatments	Control	25% N	50% N	75% N	100% N	Mean
			First season			
Container	44.1 a	31 d	41.3 b	39.4 cd	40.7 bc	**40.7 A**
Pots	43.5 a	38.6 e	41.6 b	39 d	41.1 bc	**39.3 B**
Mean	**43.8 A**	**34.8 D**	**41.4 B**	**39.2 C**	**40.9 B**	
			Second season			
Container	43.3 ab	34.6 d	41 bc	35 d	43 ab	**39.5 A**
Pots	44 a	31 e	38.3 c	38.6 c	40 c	**38.5 A**
Mean	**44 A**	**32.8 D**	**39.6 B**	**36.8 C**	**41.8 AB**	

Table 4. Effect of different mineral nitrogen rates with Rhizobium inoculation compared to 100% mineral N without inoculation and substrate systems on total leave area (cm^2/plant) of snap beans cv. Goya during (2007/2008) and (2008/2009) autumn seasons.

Treatments	Control	25% N	50% N	75% N	100% N	Mean
			First season			
Container	2007 a	759 f e	1432 c	973 e	1792 b	**1393 A**
Pots	1379 c	680 g	1161 d	1141 d	1114 d	**1095 B**
Mean	**1693 A**	**720 E**	**1297 C**	**1057 D**	**1453 B**	
			Second season			
Container	1996 a	819 g	1425 c	1302 d	1640 b	**1436.8 A**
Pots	1120 e	658 h	1078 e	952 f	1103 e	**982.4 B**
Mean	**1558.3 A**	**739 E**	**1251.6 C**	**1127.3 D**	**1371.6 B**	

Early and total yield (g/m^2)

Results presented in Tables (5 and 6) showed that the effect of different nitrogen rates and substrate systems on early and total yield (kg/m^2) of snap bean plants grown under condition of N_2-fixing for both seasons.

For both seasons, using container system significantly increased early yield and total yield of snap bean plants in comparison with using pots system.

Regarding the effect of nitrogen rates on early yield and total yield, data showed that using control gave the highest early yield and total yield, followed by 100% N. The differences between the mentioned treatments and the other treatments were significant. The lowest early and total yield along the first and second season was obtained by using 25% N.

Regarding the effect of interaction, data illustrated that the control container recorded the highest value of early and total yield (kg/m^2) followed by 100% N combined with container system; come in the third order 50% N combined with container system.

The lowest early and total yield (kg/m^2) was obtained by 25% combined with pots system in the both seasons. These data agreed with, **Asif and Greig (1968)** who reported that 67 kg ha^{-1} nitrogen application in bean increased the grain yield. Similarly in a study reported by **(Ukkelberg *et al.,* 1964)** 60-120 kg ha^{-1} nitrogen application significantly increased the yield.

A study on the effect of nitrogen fertilizer application on the yield of common beans in Ecuador **(CIAT, 1975)** showed a positive response up to 200 and 400 kg N ha^{-1} there is evidence that N fertilization of common bean can improve nodulation and yield when N is used as starter fertilizer **(CIAT, 1995, Mitiku, 1990).**

Shibru and Mitiku (2002) found that inoculation with mixed granular rhizobial inoculate and use of starter nitrogen fertilizer significantly improved yield. Bacteria inoculation exerted a significant and positive effect on pod number per plant and grain yield **Numan and Yilmaz (2005).**

Table 5. Effect of different mineral nitrogen rates with Rhizobium inoculation compared to 100% mineral N without inoculation and substrate systems on early yield (g/m2) of snap beans cv. Goya during (2007/2008) and (2008/2009) autumn seasons.

Treatments	Control	25% N	50% N	75% N	100% N	Mean
			First season			
Container	12100 a	8682 e	8770 e	9790 d	11454 b	**10159 A**
Pots	10540 c	3993 h	6064 g	6289 g	8169 f	**7011 B**
Mean	**11320 A**	**6338 E**	**7417 D**	**8039 C**	**9812 B**	
			Second season			
Container	12826 a	9203 e	9296 e	103777 d	12141 b	**10769 A**
Pots	11173 c	4233 h	6428 g	6666 g	8659 f	**7432 B**
Mean	**11999 A**	**6718 E**	**7862 D**	**8521 C**	**10400 B**	

Table 6. Effect of different mineral nitrogen rates with Rhizobium inoculation compared to 100% mineral N without inoculation and substrate systems on total yield (g/m^2) of snap beans cv. Goya during (2007/2008) and (2008/2009) autumn seasons.

Treatments	Control	25% N	50% N	75% N	100% N	Mean
			First season			
Container	7963 a	3776 e	6428 c	5649 d	6863 b	**6136.2 A**
Pots	3848 e	2577 g	3402 f	3293 f	3807 e	**3385.3 B**
Mean	**5905 A**	**3176 E**	**4915 C**	**4471 D**	**5335 B**	
			Second season			
Container	7512 a	3562 e	6064 c	5329 d	6475 b	**5788.6 A**
Pots	3630 e	2431 g	3210 f	3106 f	3591 e	**3193.9 B**
Mean	**5571 A**	**2996.6 E**	**4637.3 C**	**4218 D**	**5033.3 B**	

Chemical properties

Total soluble solids (T.S.S. %)

Results presented in Table (7) show the effect of different nitrogen rates and substrate systems on total soluble solids (T.S.S. %) of snap bean plants grown under condition of N_2-fixing. As for the two experimental seasons, data showed that container system increased total soluble solids in comparison with using pots system.

Using the control and 100% N treatment gave the highest TSS. The 25% N treatment gave the lowest values.

Regarding the interaction effect, data showed that there is no significant among controls, 100%, 75% and 50% N combined with container and 100% N combined with pots followed by control combined with pots

While the lowest record was obtained by 25% N treatment combined with container and 25%, 50% and 75% N treatment combined with pots in both seasons.

Pod protein percentage

Data in Table (8) illustrated the effect of different nitrogen rates and substrate systems on pod protein percentage of snap bean plants grown under condition of N_2-fixing. Regarding the effect of substrate system, data showed that the container system recorded higher significant pod protein percentage than pot system.

Regarding the effect of nitrogen rates on pod protein percentage, data showed that using control and 100% N gave the highest pod protein percentage, followed by 75%, 50% and 25 % N without significant difference among them, in both seasons.

Concerning the interaction effect, the highest values of pod protein percentage were recorded by container control and 100% N combined with container. Data showed that there were no significant among (75% and 50% N combined with container) and (control, 25 and 100% N combined with pots) in the both seasons.

Treatment 25% N combined with container recorded the lowest values of pod protein percentage in the both seasons.

Total-N content

The results presented in Table (9) illustrated the effect of different nitrogen rates and substrate systems on total-N content of pods of snap bean plants grown under condition of N_2-fixing. Regarding the effect of substrate system, data showed that the container system recorded the higher total-N content compared to the pots system in both season.

On the other hand, regarding the effect of nitrogen rates on total-N content of pods, data showed that using control and 100% N treatment gave the highest total-N content of pods, followed by 75% and 50% N the treatment was no significant between them, in both seasons. The lowest total-N content the first and second season was obtained by using 25% N.

Concerning the interaction effect, the highest values of total-N content of pods were recorded by container control and 100% N combined with container. Data showed that there is no significant between (75% and 50% N combined with container) and (control, 50% and 100% N combined with pots) in both seasons.

Treatment 25% N combined with (container and pot) systems recorded the lowest values of total-N content of pods in both seasons.

Batra *et al*., (1992) found that nitrate reductase activity in leaves and total N uptake were highest with higher rates N. Concerning the effect of inoculation of Rhizobium on total carbohydrates, nitrogen and protein in dry seeds, the highest values was obtained by this treatment compared with uninoculated treatment. Similar results were reported by **Subba-Rao (1976); Alexander (1978); Moawad *et al.,* (1988) and Kanaujia *et al.,* (2000).**

Hungria and Neves (1987) found that Phaseolus vulgaris cv. always benefited from N applications. Among the various N sources supplying the seeds, the most important one was the fixed N translocated directly from nodules or after a rapid transfer through leaves, representing 60-64% of the total N incorporated into the seeds. Nitrogen application exerted a significant and positive effect on raw protein proportion **Numan and Yilmaz (2005).**

Table 7. Effect of different mineral nitrogen rates with Rhizobium inoculation compared to 100% mineral N without inoculation and substrate systems on total soluble solids (T.S.S. %) of snap beans cv. Goya during (2007/2008) and (2008/2009) autumn seasons.

Treatments	Control	25% N	50% N	75% N	100% N	Mean
			First season			
Container	5.9 a	5.4 c	5.9 ab	5.9 a	6 a	**5.8 A**
Pots	5.6 b	5.2 c	5.4 c	5.3 c	5.8 ab	**5.5 B**
Mean	**5.8 AB**	**5.3 C**	**5.6 B**	**5.6 B**	**5.9 A**	
			Second season			
Container	6.3 a	5.7 c	6.2 ab	6.3 a	6.3 a	**6.1 A**
Pots	6 b	5.5 c	5.7 c	5.6 c	6.1 ab	**5.8 B**
Mean	**6.1 AB**	**5.6 C**	**6 B**	**6 B**	**6.2 A**	

Table 8. Effect of different mineral nitrogen rates with Rhizobium inoculation compared to 100% mineral N without inoculation and substrate systems on pod protein percentage (g/100g) dry weight of pods of snap beans cv. Goya during (2007/2008) and (2008/2009) autumn seasons.

Treatments	Control	25% N	50% N	75% N	100% N	Mean
			First season			
Container	22.2 a	16.2 e	18.7 bc	18.6 bc	21.9 a	**19.5 A**
Pots	19.4 b	19.4 b	17.8 cd	16.9 de	18.7 bc	**18.4 B**
Mean	**20.8 A**	**17.8 B**	**18.2 B**	**17.7 B**	**20.3 A**	
			Second season			
Container	23.6 a	17.2 e	19.8 bc	19.7 bc	23.2 a	**20.7 A**
Pots	20.6 b	20.5 b	18.9 cd	17.9 de	19.8 bc	**19.6 B**
Mean	**22 A**	**18.8 B**	**19.3 B**	**18.8 B**	**21.5 A**	

Table 9. Effect of different mineral nitrogen rates with Rhizobium inoculation compared to 100% mineral N without inoculation and substrate systems on total-N content percentage (g/100g) dry weight of pods of snap beans cv. Goya during (2007/2008) and (2008/2009) autumn seasons.

Treatments	Control	25% N	50% N	75% N	100% N	Mean
			First season			
Container	3.5 a	2.5 d	2.9 b	2.9 b	3.5 a	**3.1 A**
Pots	3.1 b	2.4 d	2.9 b	2.7 c	2.9 b	**2.8 B**
Mean	**3.3 A**	**2.4 C**	**2.9 B**	**2.8 B**	**3.2 A**	
			Second season			
Container	3.7 a	2.6 d	3.1 b	3.1 b	3.7 a	**3.2 A**
Pots	3.3 b	2.6 d	3.1 b	2.8 c	3.1 b	**3 B**
Mean	**3.5 A**	**2.6 C**	**3.1 B**	**3 B**	**3.4 A**	

Nodulation and N_2- fixing activity

Number of nodules

Results presented in Table (10) show the effect of different nitrogen rates and substrate systems on number of nodules of snap bean plants under condition of N_2-fixing. For the two experimental seasons, container system increased number of nodules in comparison with using pots system. On the other hand, regarding the effect of nitrogen rates on number of nodules, data showed that using 50% N gave the highest number of nodules, followed by 75% N treatment, followed by 25% N , while there are no nodules in control and 100% N treatment in both season.

Concerning the interaction effect, the highest values of number of nodules were recorded by 50% N treatment combined with container, followed by 50% N treatment combined with pots. The lowest records of number of nodules were recorded by 25% N treatment combined with pots in both seasons. While there are no nodules in control and 100% N treatment container and pots in both season.

Nitrogenase activity

Data in Table (11) illustrated the effect of different nitrogen rates and substrate systems on nitrogenase activity of snap bean plants grown under condition of N_2-fixing. Data showed that using container system increased nitrogenase activity in comparison with using pots system. On the other hand, regarding the effect of nitrogen rates on nitrogenase activity, data showed that using 50% N gave the highest nitrogenase activity, followed by 75% N treatment, followed by 25% N treatment, while there are no nitrogenase activity in control and 100% N treatment in both season.

Referring the effect of interaction, the highest values of nitrogenase activity were recorded by 50% N treatment combined with container, followed by 50% N treatment combined with pots. The lowest records of nitrogenase activity were recorded by 25%N treatment combined with pots in both seasons. While there are no nitrogenase activity in control and 100% N treatment container and pots in both season.

Similar results were obtained according to **Batra *et al.*, (1992)** found that nodulation and nitrogenase activity were increased by up to 20 p.p.m N but decreased at higher rates. **Isoi and Yoshida (1991)** suggested that low N fixation in P. vulgaris may be related to the absence of hydrogenases in *Rhizobium leguminosarum bv. ph*
The positive significant correlation between nodule number and grain yield agrees with the findings of **CIAT (1995)**. However, use of only modest amount of N without inoculation gave significantly higher nodule number than the control.

Table 10. Effect of different mineral nitrogen rates with Rhizobium inoculation compared to 100% mineral N without inoculation and substrate systems on number of nodules of snap beans cv. Goya during (2007/2008) and (2008/2009) autumn seasons.

Treatments	Control	25% N	50% N	75% N	100% N	Mean
			First season			
Container	00 g	32 e	57 a	45 c	00 g	**27 A**
Pots	00 g	22 f	49 b	40 d	00 g	**22 B**
Mean	**00 D**	**27 C**	**53 A**	**42 B**	**00 D**	
			Second season			
Container	00 g	34 e	61 a	47 c	00 g	**28 A**
Pots	00 g	24 f	52 b	42 d	00 g	**23 B**
Mean	**00 D**	**29 C**	**57 A**	**45 B**	**00 D**	

Table 11. Effect of different mineral nitrogen rates with Rhizobium inoculation compared to 100% mineral N without inoculation and substrate systems on nitrogenase activity (acetylene reduction activity, ARA) of snap beans cv. Goya during (2007/2008) and (2008/2009) autumn seasons.

Treatments	Control	25% N	50% N	75% N	100% N	Mean
			First season			
Container	00 g	17.7 e	31.4 a	24.5 c	00 g	**14.7 A**
Pots	00 g	12.3 f	27 b	21.8 d	00 g	**12.2 B**
Mean	**00 D**	**15 C**	**29 A**	**23 B**	**00 D**	
			Second season			
Container	00 g	18.8 e	33.4 a	26 c	00 g	**15.6 A**
Pots	00 g	13 f	28.5 b	33 d	00 g	**12.9 B**
Mean	**00 D**	**16 C**	**31 A**	**24.5 B**	**00 D**	

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