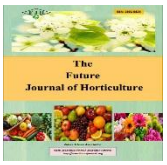

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Response of Yield and Quality of Snap Bean to Nitrogen, Biogas and Seed Inoculation

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Abstract: This work was conducted at a private farm, Alharsha district, Zawia Region, Libya in two summer seasons of 2020 & 2021, to study the effect of Rhizobium Bacteria, nitrogen and biogas fertilizers on growth, chemical contents and yield of snap bean. The experiment included 6 treatments, i.e. 2 seed inoculation (with and without nitrogen fixing bacteria of genus *Rhizobium*), and 3 rates of nitrogen (100 kg N/ha), organic biogas (80 kg/ha) and (control) without any addition. Treatments were arranged in split – plot design with three replicates, where the treated seeds with rhizobium and untreated seeds were assigned in main plots and the three treatments of O (control), nitrogen level (100 kg/ha) and biogas rate (80 /ha) were distributed in sub plots. Results indicated that Rhizobium inoculation, being the most effective treatment on plant growth characters, physical pod character, pod yield and its attributes, as well as the nutritive value of snap bean pods in both seasons. Nitrogen fertilizer caused a significant increase in vegetative growth parameters, physical pod character and pod yield and its components as well as the nutritive value of snap bean. This result is true in both growing seasons. The followed result of this treatment was the biogas treatment compared to the un-fertilizer treatment (control). The interaction effect of rhizobium inoculation with organic biogas fertilizer, followed by the treatments of rhizobium + nitrogen fertilizer and nitrogen fertilizer alone without rhizobium inoculation, respectively, caused an increases in vegetative growth parameters, physical pod character and pod yield and its attributes, as well as the chemical constituents of snap bean pod cv. Contender.

Key words: Nitrogen and biogas fertilizer, Seed inoculation, Snap bean.

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INTRODUCTION

Snap bean (*Phaseolus vulgaris* L.) is among the most important vegetable grown in Libya for local consumption after peas crop. It is an important source of proteins, minerals and vitamins for human race **(Elkhatib, 2009).**

Rhizobium bacteria inoculation to plays a fundamental role in is fixing air nitrogen which increased the soil nitrogen content and improved the fertility (**Poth *et al.*, 1986 and Abu Khouder *et al.*, 2019).**

Many investigates illustrated that rhizobium inoculation on legumes increased their growth and pod yield **(Derib, 2014 & Solaiman and Khondaker, 2002).**

Biogas fertilizer is one of an organic fertilizer that can be used to improve the fertility of soils and to improve the soils fertility and crop productivity **(Kushelaf *et al.*, 2014 and Abou El -Salehein *et al.*, 2019)** concluded that biogas fertilizer increased growth, chemical composition and pod yield of pea.

Nitrogen is an indispensable elementary constituent of numerous organic compounds of general importance in plant parts, and thus increased fresh and dry weight of different parts of plant **(Edmond *et al.*, 1981)**, stated that nitrogen fertilizer increased growth, chemical composition and pod quality of snap bean.

The aim of this work was to investigate the effect of rhizobium inoculation, biogas and nitrogen fertilizers on growth, chemical contents and pod yield and quality of green pods.

MATERIALS AND METHODS

This study was conducted at a private farm, Alharsha district, Zawia Region, Libya in the two summer successive seasons of 2020 and 2021, to investigate the effect of Rhizobium Bacteria, nitrogen and biogas fertilizers on growth, chemical contents and yield of snap bean cv. Contender. The experiment included 6 treatments, i.e. 2 seed inoculation (with and without nitrogen fixing bacteria of genus *Rhizobium*), and 3 rates of nitrogen (100 kg N/ha), organic biogas (80 kg/ha) and (control) without any addition.

Bean seed inoculation were done with the symbiotic N-fixing bacteria of genus Rhizobium (*Rhizobium phaseolli*) by coating the seeds at the rate of 20 g. of Oqadin / kg of seeds using staking substance (5% Arabic gum) just before sowing and then were directly sown on one side of the row.

The physical and chemical analysis of the soil of experiment and water irrigation carried out according to the methods of **Black (1982)** and the values are tabulated in Tables (1, 2).

Table 1. The physical and chemical properties of the experimental soil

Properties		Value
Physical analysis		
Sand	(%)	83.5
Silt	(%)	5.6
clay	(%)	10.9
Soil texture		Sandy loam
Chemical analysis		
Calcium carbonate (Ca CO_3, g/kg		7.9
Organic matter (g/kg)		2.17
pH		7.6
EC (Electric conductivity, dS /m)		1.27
Soluble Cations (mmol/L)		
Calcium (Ca^{++})		10.57
Magnesium (Mg^{++})		3.53
Sodium (Na^{+})		2.07
Potassium (K^{+})		1.87
Soluble onions (mmol/L)		
Bicarbonate (HCO_3-)		4.83
Chlorine (Cl^{-})		2.77
Sulphate ($SO4^{-}$)		3.43
Available nutrients (mg/L)		
Macronutrients		
Nitrogen (N)		86
Phosphorus (p)		8
Potassium (K)		65
Macronutrients		
Fe		9.97
Zn		3.45
Cu		2.33
Mn		6.53

Table 2. The analysis of irrigation water used

Characters	Concentration
Total Salts (ppm)	1296
Ca^{++}	13.5
Mg^{+}	16.0
Na^{+}	12.3
K^{+}	1.99
SO_4-	3.93
HCO_3-	3.03
EC (Electric conductivity, dS/m)	0.60

The experimental site was prepared and Rhizobium inoculation was done to the treatments which treated with its. The rhizobium was received from Egyptian Agriculture Ministry, Agricultural Research Centre, Egypt.

The treated and untreated seeds were sown on April 4th and 5th in 2020 and 2021, respectively.

Treatments were arranged in split – plot design with three replicates. The experiment included 6 treatments, where two seed treatments, i.e. the treated seeds with rhizobium and untreated seeds were assigned in main plots and the three treatments of O (control) nitrogen level (100 kg/ha) and biogas rate (80 /ha) were distributed in sub plots.

Nitrogen fertilizer was added in the form of ammonium sulphate (20.5 %N) and biogas are received from the unit of biogas, Faculty of Agriculture, Zagazig University. Egypt. The analysis of biogas are presented as shown in Table (3).

Table 3. The contents of biogas fertilizer

Characters	Concentration
Total (N)%	1.53
Total (p)%	0.67
Total (K)%	1.17
Total Carbon (C)%	35.5
Fe (ppm)	1553
Zn (ppm)	185
Mn (ppm)	179.75
EC (1 : 10) (dS/m)	0.60
pH	7.1
Moisture content (%)	67
Ash	18

All treatments were received 100 kg/ha calcium superphosphate (16% P_2 O_5) and 100 kg/ha potassium sulphate (48% k_2O).

The area of sub-plot was 8.4 m^2 (4 ridges of 3.5 m long and 0.6 m width). Seeds were sown on one side of ridge at 10cm a part, and one guard row was left without planting between each two plots.

The different amounts of fertilizers were applied at two equal portions, at seeds sowing and four weeks after sowing.

The other cultural procedures of growing bean were practiced as usually followed in the commercial production of green pod yield.

Data Recorded

Plant Growth Characters

At 8 weeks after sowing, height of plant (cm), leaves number, fresh and dry weight of whole plant (g) were recorded, randomly chosen five plants from each treatment.

At harvest time, fresh pod yield of each experimental plot was harvest and the following data were recorded as follows:

Pod Physical and Green Pod Yield

It was calculated from weight and number of all harvested pods through whole harvesting season and number of plant per plot.

Twenty pods were randomly taken from each treatment in all replicates at the second harvesting in both growing seasons and mean pod length, diameter (cm) and fresh weight /pod (g) were recorded.

The average number of pods per plant, total green pod yield per plant (g) and per hectare (ton) were calculated.

The Nutritive Value of Pods (Pod Chemical Constituents)

- Total N, P and K percentage were determined as the methods of **Cottenie *et al.* (1982).**
- Total carbohydrates percentage: It was determined according to the methods of **Michel *et al.* (1956).**
- Fibers: it was determined according to the methods described by **A.O.A.C. (1984)**.

Statistical Analysis

The obtained data were statistically analyzed using SAS Software program (**SAS Institute, 2004)**. The means of treatments were conducted using the Procedure of Least Significant Difference (LSD) at p = 0.05 level as described by **Snedecor and Cochran (1980).**

RESULTS AND DISCUSSION

Vegetative Growth Characters

Effect of Rhizobium Inoculation

All treatments had an increase on the vigor growth parameters, i.e. height of plant (cm), leaves number /plant, fresh and dry weight of bean plant (g), in both seasons of 2020/2021 and 2021/2022 as shown in Table (4).

Rhizobium inoculation, being the most effective treatment on growth characters of snap bean plants in both seasons compared to the control treatment (without inoculation).

Regarding the effect of rhizobium on legume plants, **Elkhatib (2009)** demonstrated that rhizobium bacteria plays an important role on air nitrogen fixation, that improved the growth of plant and then increased plant growth parameters.

The obtained results are confirmed with the results of **Mishra *et al.* (2010) and El- Seifi *et al.* (2013)** who concluded that rhizobium inoculation increased plant growth characters of legumes plants.

Effect of Nitrogen and Biogas Fertilizers

Data tabulated in Table (4) indicate that nitrogen fertilizers caused a significant increase in vegetative growth parameters of snap bean. This result is true in both growing seasons. The followed result of this treatment was the biogas treatment compared to the un-fertilizer treatment (control).

According the effect of nitrogen and biogas fertilizers on legume plants for increasing plant growth characters, **Chen (2019)** illustrated that nitrogen is an essential element that play an important role in plant life cycle and too is an essential constituent of protein (build from amino acids that involves in catalization of chemical responses and transportation of electrons). In addition, nitrogen plays a most important role in different physiological processes.

Moreover, biogas is type from organic manure that has release the elements by micro-organisms from the complex form to easier absorption by roots of snap bean plant, then increased the vigor growth

of different plant parts [10]. Those results could be explained by the findings obtained of **Asmaa *et al.* (2013) and Abou El –Salehein *et al.* (2019).**

The Interaction Effect of Rhizobium Inoculation and Fertilizer

Data recorded in Table (4) demonstrate that plant growth parameters of snap bean plants caused a highest value in response of treating bean plants with the treatment of rhizobium inoculation + biogas fertilizer, followed by the treatments of rhizobium + nitrogen fertilizer as well as, nitrogen fertilizer without rhizobium inoculation, respectively. These results are confirmed with those recorded by **(Su *et al.*, 2008 and Kushelaf *et al.*, 2014).**

Table 4. Effect of rhizobium inoculation and nitrogen and biogas fertilizers and their combined effect on vigor growth of snap bean during 2020 and 2021 seasons

Treatments	Height of plant (cm)		leaves number		Fresh weight /plant (g)		Dry weight/plant (g)	
	2020 season	2021 season	2020 season	2021 season	2020 season	2021 season	2020 season	2021 season
Rhizobium Inoculation (A):								
Without	27.1	26.87	14.90	14.60	80.93	80.90	19.95	15.97
With	28.7	28.87	16.37	16.43	85.92	85.90	17.01	17.02
LS.D (0.05)	1.2	1.88	1.10	1.17	3.67	3.81	0.13	0.17
Fertilizer (B):								
O (Control)	25.95	25.9	13.95	14.60	77.80	77.78	14.74	14.73
Nitrogen	28.90	28.85	16.30	16.20	85.83	85.80	17.13	17.17
Biogas	28.85	28.85	16.95	16.45	86.64	86.39	17.58	17.58
LS.D (0.05)	0.03	0.09	0.55	0.11	0.71	0.63	0.36	0.27
Interaction:								
O	25.4	25.1	13.2	12.9	75.17	75.15	14.12	14.16
Without Nitrogen	28.7	28.5	16.1	15.7	84.51	84.47	17.10	17.14
Rhizobium Biogas	27.2	27.0	15.4	15.2	73.11	83.07	16.63	16.61
O	26.5	26.7	14.7	14.9	80.43	80.40	15.35	15.30
With Rhizobium Nitrogen	29.1	29.2	16.5	16.7	87.15	87.13	17.16	17.19
Biogas	30.5	30.7	16.9	17.7	90.17	90.11	18.53	18.55
LS.D (0.05)	0.30	0.20	0.30	0.2	2.15	2019	0.03	0.03

Physical Pod and Green Pod Yield

Effect of Rhizobium Inoculation

Application of rhizobium inoculation of seed snap bean showed a consistent effect on physical pod characters (length, diameter and fresh weight of pod) and pods number /plant, average pod weight and total green pod yield / hectare (Tables,5 - 6). This treatment is true in both seasons compared to the untreated without rhizobium inoculation.

Respecting the role of rhizobium in increasing pod yield of snap bean, **Elkhatib (2009)** stated that rhizobium inoculation of seed pea increased the pod yield of pea that may be to their important role in fixing air nitrogen and building different tissues, as well as increased number of pods and their weight, then consequently increased the pod yield. Similarly, results were obtained by **Fekry et al., (2003) & Yadegari and H. Rahmani (2010).**

Effect of Nitrogen and Biogas Fertilizers

Data tabulated in Tables (5 - 6) show that application of nitrogen fertilizer significantly influenced the values of studied characters, i.e. physical pod characters, pods number /plant, average pod weight

and green pod yield / hectare. In this concern, the highest values were obtained by soil application of nitrogen fertilizer, followed by organic biogas fertilizer during both seasons.

Regarding the effect of nitrogen and biogas fertilizers in increasing the pod yield of snap bean, **Bloom (2015)** pointed out that nitrogen plays an important role in various bio-chemical processes, such as promotes and stimulates pods formation, then consequently increased pod yield. Moreover, biogas fertilizers are used as an organic fertilizer that his role in environment friendly and save application which provide the plants with nutrients within release the nutrients by promoting micro-organisms in soil and translocate to plants, then increased pod yield and its attribute **(Fekry *et al.*, 2003; Yadegari and Rahmani, 2010) and Abou El –Salehein *et al.*, 2019).**

This results are confirmed with those found by **Ishaq (2002)** who working on peas with nitrogen and bio-fertilizers, and **Ismail (2002)** on pea too with bio-fertilizers.

Table 5. Effect of rhizobium inoculation and nitrogen and biogas fertilizers and their combined effect on physical pod characters of snap bean during 2020 and 2021 seasons

Treatments	Length of pod (cm)		Diameter of pod (cm)		Fresh pod weight/plant (g)	
	2020 season	2021 season	2020 season	2021 season	2020 season	2021 season
without	11.67	11.70	0.80	0.90	71.63	71.58
With rhizobium	13.22	13.24	1.07	1.10	81.39	81.37
LS.D (0.05)	1.25	1.19	0.11	0.12	7.39	8.27
0	11.16	11.18	0.75	0.85	66.35	66.40
Nitrogen	13.10	13.14	1.00	1.10	80.84	80.70
Biogas	13.08	13.07	1.05	1.05	82.34	82.32
LS.D (0.05)	0.15	0.13	0.12	0.03	1.27	1.32
O	10.17	10.19	0.7	0.8	61.57	61.63
Without N	12.63	12.67	0.9	1.0	78.17	78.05
Biogas	12.22	12.24	0.8	0.9	75.14	75.10
O	12.15	12.17	0.8	0.9	71.13	71.17
With N	13.57	13.60	1.1	1.2	83.50	83.39
Biogas	13.93	13.91	1.3	1.2	89.53	89.55
LS.D (0.05)	0.08	0.09	N.S	N.S	2.63	2.75

Table 6. Effect of rhizobium inoculation and nitrogen and biogas fertilizers and their combined effect on pod yield and its components of snap bean during 2020 and 2021 seasons

Treatments	Number of pods/plant		Total green pod yield/plant(g)		Total green pod yield/ha (ton)	
	2020 season	2021 season	2020 season	2021 season	2020 season	2021 season
without	27.48	28.45	74.173	74.166	5.876	5.910
With rhizobium	31.59	31.07	82.873	82.850	6.993	6.994
LS.D (0.05)	3.41	2.36	6.15	6.35	0.013	0.014
0	23.93	23.92	68.05	67.97	4.965	4.975
Nitrogen	31.84	31.83	83.75	83.79	7.12	7.16
Biogas	32.08	32.08	83.77	83.76	7.22	7.22
LS.D (0.05)	0.12	0.14	0.11	0.10	0.09	0.05
O	20.59	20.63	60.96	60.85	4.15	4.22
Without N	31.53	31.47	81.35	81.39	6.91	6.96
Biogas	30.31	30.27	80.21	80.26	6.57	6.55
O	27.27	27.22	75.14	75.09	5.78	5.73
With N	32.15	32.19	86.15	86.19	7.33	7.36
Biogas	33.85	33.81	87.33	87.27	7.87	7.89
LS.D (0.05)	1.07	1.09	0.21	0.20	0.03	0.04

The Interaction Effect of Rhizobium and Fertilizers

The combined effect of rhizobium inoculation and fertilizer (nitrogen and biogas) on physical pod parameters and pod yield of snap bean are demonstrated in Tables (5 - 6). The results of both growing seasons revealed that the highest values of all studied parameters were obtained from the plants that were inoculated by rhizobium and fertilizer of nitrogen, followed by treatments of rhizobium inoculation with biogas fertilizer and nitrogen fertilizer only without inoculation, respectively. Comparable results were also detected by **(Abou El –Salehein *et al.*, 2019)**.

Pod Quality (Nutritive Value of Pod)

Effect of Rhizobium Inoculation

As regarding to Tables (7, 8) that the treatment of seed bean inoculation significantly increased the chemical pod constituent, i.e. N, P, K and carbohydrates compared to the untreated seeds without inoculation.

Meanwhile, this treatment caused a decreased in the percentage of fibres of bean pod.

Concerning the effect of rhizobium inoculation on legume plants, **Dawa *et al.* (2014)** illustrated that rhizobium inoculation on pea seed significantly increased the chemical contents of pods, i.e. N, P, and K percentages. In addition, **Solaiman and Khondaker (2002)** stated that the chemical contents of pea pods increased as a result of plants were inoculated with rhizobium strains. Moreover, rhizobium inoculation on bean seed significantly increased the total carbohydrates and N, P, and K percentages **(Yadegari and Rahmani, 2010)**.

These results are followed the same patterns of **(Derib, 2014; Donald, 2016 & Farid and Navabi, 2015)** who concluded that rhizobium inoculation increased the legumes plants.

Effect of Nitrogen and Biogas Treatments

The illustrated data in Tables (7 and 8) show that the treatment of nitrogen fertilizer, followed by biogas treatment caused a highest increase in N, P, K and carbohydrates.

Meanwhile, biogas treatment significantly decreased the percentage of fibers on bean pod compared to the treatments of nitrogen fertilizer and untreated plants.

In this concern, nitrogen has an important role and organic biogas too in increasing pod quality of bean, may be attributed to their effects on growth parameters (Table, 4) and pod yield attributes (Tables, 5 and 6).

These results are in confirming with those recorded by **Abu Khouder *et al.* (2019)** who working with nitrogen fertilizer and **Abou El –Salehein *et al.* (2019)** who working with biogas on pea plants.

The Combined Effect Between Seed Inoculation and Nitrogen and Biogas on Pod Quality

Data recorded in Tables (7 and 8) conclude that N, P, K and total carbohydrates in bean pod caused a highest value in case of treating snap bean with the treatment of rhizobium inoculation + biogas treatment, followed by seed inoculation with rhizobium inoculation + nitrogen fertilizer treatment, respectively. These results are in a good pattern with those recorded by **Abou El –Salehein *et al.* (2019)**.

Table 7. Effect of rhizobium inoculation and nitrogen and biogas fertilizers and their combined effect on N, P and K percentages of pod of snap bean during 2020 and 2021 seasons

Treatments	N%		P%		K%	
	2020 season	2021 season	2020 season	2021 season	2020 season	2021 season
without	3.26	3.24	0.28	0.28	3.00	3.05
With rhizobium	3.55	3.53	0.35	0.36	3.34	3.33
LS.D (0.05)	0.23	0.25	0.07	0.05	0.02	0.02
0	3.00	2.98	0.25	0.24	2.64	2.6.3
Nitrogen	3.66	3.63	0.35	0.35	3.46	3.46
Biogas	3.57	3.56	0.36	0.37	3.42	3.43
LS.D (0.05)	0.08	0.06	0.03	0.02	0.02	0.02
O	2.87	2.83	0.22	0.23	2.47	2.51
Without N	3.59	3.55	0.32	0.31	3.37	3.41
Biogas	3.33	3.36	0.31	0.30	3.17	3.22
O	3.12	3.14	0.27	0.25	2.81	2.85
With N	3.73	3.70	0.37	0.39	3.55	3.51
Biogas	3.79	3.76	0.41	0.43	3.67	3.63
LS.D (0.05)	0.05	0.06	0.02	0.01	0.01	0.02

Table 8. Effect of rhizobium inoculation and nitrogen and biogas fertilizers and their combined effect on total carbohydrates and fibers of pod of snap bean during 2020 and 2021 seasons

Treatments	Total carbohydrates (%)		Fibers (%)	
	2020 season	2021 season	2020 season	2021 season
without	18.89	18.82	11.50	11.53
With rhizobium	21.25	21.34	10.91	10.86
LS.D (0.05)	2.17	2.12	0.42	0.36
0	16.75	16.77	12.95	12.94
Nitrogen	21.81	21.76	15.36	10.36
Biogas	21.66	21.72	10.31	10.28
LS.D (0.05)	0.13	0.14	1.43	0.05
O	15.17	15.19	13.57	13.63
Without N	21.36	21.22	10.49	10.49
Biogas	20.15	20.07	10.46	10.45
O	18.33	18.36	12.33	12.26
With N	22.26	22.30	15.22	10.23
Biogas	23.17	23.37	10.17	10.11
LS.D (0.05)	1.19	1.23	0.10	0.07

Conclusion

It could be summarized that seed inoculation with rhizobium strain + biogas fertilizer and rhizobium strain + nitrogen fertilizer treatments, respectively caused an increases in vegetative growth, pod physical characters and pod yield and quality of snap bean.

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