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The Effect of *Rhizobium leguminosarum* and *Bacillus megatherium* Inoculation and Some Chemical Fertilizers On Growth, Chemical Composition and Green Pod Yield of Peas (*Pisum sativum* L.)

A Thesis Submitted in the Libyan Academy in Partial Fulfillment of the Requirement for the Degree of
Master of Science in Botany

Prepared by :
Karima Abdullah Hmed

Supervised by :
Dr. Essam Abou El-Salehein

Prof. of Hort. Plant production Dept.
Faculty of Veterinary and Agricultural Sciences
Zawia University, Zawia

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بسم الله الرحمن الرحيم

وما أوتيتم من العلم إلا قليلا

صدق الله العظيم

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Abstract:

The field trial was conducted at private farm in Tripoli city to study the effect of biofertilizers *(Rhizobium leguminosarum* and/or *Bacillus megatherium*) and chemicals fertilizers (N & P fertilizers) treatments alone or in combination with them on vegetative growth and yield of pea *(Pisum sativum* L.) cv. Master-B and seed chemical analysis .

A highly significant effect ($p \leq 0.01$) for treatments on all traits studied was observed. All treatments produced greater yield as compared by control. The best yield (64.23) , (87.17), (21.67) was recorded for (*Rhizobium* + *Bacillus* + N &P fertilizers) treatment ; the lowest yield (56.13), (61.00), (18.88) was obtained for the control and the other treatments came in between.

The overall means of vegetative growth traits averaged 60.56± 0.52 , 26.19± 0.26 and 5.44± 0.14 for plant height ; number of leaves and dry weight of leaves, respectively . The general means of yield traits averaged 14.27± 0.22, 5.07± 0.05, 72.69± 1.77, 19.60± 0.34, 5.27± 0.04, 6.89± 0.13 and 1.38± 0.03 for the number of pods/ plant, average pod weight (g), total green pod yield / plant (g), total green pod yield / hectare (ton), the number of seeds / pod , pod length(cm) and pod diameter (cm), respectively. Also, the overall means of chemical analysis of pea seeds were 6.00± 0.07, 3.77± 0.05, 4.47± 0.07, 3.19± 0.03 and 19.96± 0.20 for Total soluble solids %, Total soluble sugars %, Ascorbic acid mg/100 gm F.W, Nitrogen % and Protein % traits respectively.

A highly significant positive correlation at level 1% ($p \leq 0.01$) was observed among different variables of pea.

It was concluded from current study that for obtaining maximum grain yield of pea, seed should be inoculated with Rhizobium + Bacillus in combination with N & P fertilizers.

Dedication

TO the pure spirit of my father,

To my love mother and my brothers who have always supporting me,

To my husband who supported me all the time,

To my children,

To all researches who are working to improve the quality of life,

and to all of them I dedicate this work.

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Introduction

The legumes and grains are by far the world's most important sources of food ; grains supply starch while legumes which include bean, Peas supply protein and fats (John,2005). Legumes are very important not only as food crops but possess high propensity to grow in depleted soils thereby serving as unique symbiotic capability with nitrogen balance in the soil is thereby presereved (Okaka *et al*., 2002).

Pea (*Pisum sativum* L.) is one of the most important leguminous vegetable crops grown in the winter season (Hassan, 1993). Pea is commonly used throughout the world in human diets and has high level of amino acids , lysine and tryptophan, which are relatively low in cereal grains and contains approximately 21-25% protein (Schatz and Endres, 2009) .

The Biofertilizers or microbial inoculants are organic products containing living cells of different types of microorganisms, which have the ability to convert nutritionally important elements from unavailable to available form through biological processes. It can be generally defined as preparation containing live or latent cells of efficient strains of nitrogen fixing and phosphates solubilizing microorganism used for treatment of seed (Vessey, 2003).

They are composting the area with the objective of increasing the number of such microorganisms and accelerate microbial process to augment the extant of the availability of the nutrient in a form which can easily assimilated by plant (Subba – Rao , 1986) .

The Rhizobium *(Rhizobium leguminosarum*) as fertilizer in pulses could fix 50-200 kg of N /ha/season and is able to meet 80-90 % of the crop requirement for nitrogen . Inoculation of these crops was found to

increase the crop yield by about 10 – 15 % under farm conditions (Khurana and Dudeja , 1997).

Bacillus megatherium var. *phosphaticum* bacteria (phosphate solubilising bacteria) uses a biofertilizer for dissolving and releasing phosphorus element from the complex compounds to simple compounds and plants becomes strong and have a good growth (Elsoni and Osman , 2011) .

Minerals fertilization has been reported to be one of the important factors limiting growth vigour and yield of pea. Among some benefits , nitrogen helps plants to grow quickly , while also increasing the production of seed and fruits and bettering the quality of leaf and forage crops. Nitrogen is also considered as most important nutrient for crop to activate the metabolic activity and transformation of energy chlorophyll and protein synthesis , the substance that give plants their green color, and also aids in photosynthesis (Edmond *et al*. , 1981 and De, 1993). Also, phosphorus play a key role in the photosynthesis process , plays a vital role in a variety of the things needed by plants and it supports the formation of oils, sugars and starches .

The transformation of solar energy into chemical energy is also aided by phosphorus , as well as, development of the plant and the ability to withstand stress. Additionally , phosphorus encourages the growth of roots and promotes flowering (Edmond *et al*., 1981) .

Biological N fixation is cheaper means of improving soil fertility and productivity. The use of biological N fixation provides continuous supply of N for plant growth and add organic matter to the soil (Yakubu *et al*.,2010).

Objectives :

To determine the effects of different biological fertilization (bio-fertilizer); minerals fertilizers N & P and their interaction on yield , growth traits and chemical composition of Pea (*Pisum sativum L.*).

Literatures Review

A-Effect of biofertilizers or microorganisms on legumes:

1-Vegetative growth :

Biofertilizers are the biologically active products or microbial inoculants of bacteria and fungi which may help biological nitrogen fixation, phosphorus releasing and many elements for the benefit of plants (Subba Rao , 1984).

Abou EL- Salehein and Nasr (1994) reported that Rhizobium inoculation increased the growth of pea plant. Moreover , Erman *et al*. (2009) observed that pea inoculation treatment with Rhizobium for two years had a significant effect on the plant height , number of branches, root and shoot dry weight and number of nodules in both years of the study

Fatima *et al*. (2006) showed that there was a significant effect of *Rhizobium leguminosarum* strains (TAL-377, TAL-379 and TAL-102) alone and in combination with phosphorus on plant growth of soybean (*Glycine max*) ; survival of *Rhizobium* and phosphorus solubilizing bacteria. A mixture of effective strains with phosphorus is a promising way for enhancing the growth of legume crops.

Rather *et al*.(2010) studied the effect of bio-fertilizers *Rhizobiu* , *Azobacter* and Phosphate Solubilising Bacteria (PSB) application on growth of field pea *(Pisum sativum* L.) the co-inoculation of all the three bio-fertilizers i.e. Rhizobium , Azobacter and PSB produced significantly higher growth characters as compared to control treatment and when inoculated them individually. The treatment of *Rhizobium*+ *Azobacter* + PSB gave highest growth in terms of plant height (45.26cm), number of leaves/plant (13.33), number of branches/plant (4.20), number of nodules/plant (38.46) fresh weight and dry weight of nodules (562.34 and 122.62 mg, respectively).

Jitender (2011) working on Pea (*Pisum sativum L.*), reported that the application of 120 P_2O_5/ha with Rhizobium inoculation significantly increased the plant height, number of leaves /plant , number of nodules per plant , fresh weight of nodules per plant and dry weight of nodules/ plant followed by 100 kg/ha phosphorus application with Rhizobium inoculationof pea seeds.

Ravikumar (2012) noticed that the inoculated plants with Rhizobium possessed greater height , greater fresh weight , greater number of roots , nodules , greater number of leaves , shoots , pods , over their respective controls.

Messele *et al*. (2012) reported that inoculation of *Sinorhizobium ciceri* + *Pseudomonas* sp. with 18/20 kg NP ha^{-1} as urea and DAP increased nodule number , nodule dry weight , nodule volume and dry matter by 271.59 % , 220 % , 241.97 % and 181.40 % ,respectively over uninoculated (control) at mid flowering stage .

Abbasi *et al*.(2013) observed that in all treatment compounds , total dry matter increased slowly until 25 days after sowing , from 25 till 105 days after sowing increased rapidly. Then, decrease from 105 till 115 days after sowing (harvest time). Maximum of total dry matter (374 gr/ m^2) belonged to the higher nitrogen rates (75 kg urea/ha) and seed inoculation , while the minimum of it was in control treatment. Plant height , number of pod per plant , grain 100 weight , grain yield , number and weight of nodules per plant were significantly affected by nitrogen rates and seed inoculation. Comparison of means showed that maximum of grain yield (1276.78 kg/ha) and number of pod per plant (32.48) was obtained in the high nitrogen rates. Increasing of nitrogen rates up 50 kg/ha increased number and weight of nodules per plant but, it decreased in application of 75 kg N/ha. On the other hand, minimum values of previous traits were obtained in control treatment.

2- Crop production and its components:

Abou El-Salehein and Nasr (1994) showed that *Rhizobium* inoculation increased the chemical composition and green pod yield of pea plant.

The *Rhizobium* as fertilizer in pulses could fix 50-200 kg of N/ha/season and able to meet 80-90% of the crop requirement for nitrogen. Inoculation in these crops was found to increase the crop yield by about 10-15% (Khurana and Dudeja, 1997) .

Moreover, Erman *et al*. (2009) reported that pea inoculation treatment with Rhizobium had significant effect on the seed and biomass yield , number of pods and nitrogen and phosphorus contents of pea seeds in both years. Crop productivity can be increased by the application of chemical, organic and biological fertilizers (Elsheikh *et al*., 2009)

Rather *et al* .(2010) conducted a field experiment to find out the effect of bio-fertilizers *Rhizobium* , *Azobacter* and phosphate solubilising bacteria (PSB) application on yield and economics of field pea (*Psium sativum* L.)the co-inoculation of all the three bio-fertilizers i.e. *Rhizobium* , *Azobacter* and PSB produced significantly higher growth characters as compared to absolute control and when inoculated them individually . the yield attributes like pod length , number of pods / plant , number of seeds / pod and 1000 grain weight (g) and yield of grain and straw of pea increased by co-inoculation of bio-fertilizers and were highest for the treatment in which *Rhizobium* , *Azobacter* and PSB were co-inoculated . More over the co-inoculation of *Rhizobium* and PSB also gave beneficial results in respect to other treatments .

In addition , EL-Soni and Osman (2011) noticed that inoculation of pigeon pea with *Rhizobium* significantly increased pigeon pea seed yield , 100-seed weight , moisture content , ash , fat , fiber and protein . They added also, that

the previous characters significantly increased with *Bacillus megatherium* var. *phosphaticum* strain. Moreover , when the both

treatments were used , the results were better compared to using treatment alone .

Rugheim and Abdelgani (2011) found that phosphate solubilizing bacteria individually significantly increased faba bean yield and its quality.

Ravikumar (2012) observed that the inoculated plants with Rhizobium possessed greater pods , length of pods and seed weight , over their respective controls.

3-Seeds yield and chemical composition :

Abou El-Salehin and Nasr (1994) showed that *Rhizobium* inoculation increasing green pod yield and its quality and nutritive value of pea plant.

The increase in protein content due to biological fertilizers was reported by Elsheikh (2001).

Erman *et al*.(2009) reported that pea inoculation treatment with *Rhizobium* had a significant effect on the seed and biomass yield , number of pods and nitrogen and phosphorus contents of pea seeds in both years .

El-Soni and Osman (2011) found that pigeon pea plants were either inoculated with five introduced or locally isolated *Rhizobium* or *bradyrhizobium* strains each alone or with *Bacillus megatherium* var *phosphaticum* strain (BMP) alone and combination of each rhizobial strain with *Bacillus megatherium* var *phosphaticum* strain (BMP). In addition to control for comparison. Inoculation with *Rhizobium* , BMP, *Rhizobium* + BMP significantly ($p \leq 0.05$) increased pigon peas seed yield, 100-seed weight , moisture content, fat, fiber and protein

Rugheim and Abdelgani (2011) observed that phosphate solubilizing bacteria individually significantly increased faba bean yield and its quality.

Ravikuumar (2012) reported that the inoculated plants with Rhizobium possessed greater seed weight , over their respective controls.

Abdalla *et al*. (2013) showed that effects of inoculation with biop-fertilizer (*Rhizobium* and/or *Bacillus megatherium* var. phosphaticum) and chemicals fertilizers (phosphorus and nitrogen fertilization) alone or in combination of them for chickpea variety Bergag were significantly ($p \leq 0.05$) affected increased yield , 100 seed weight, ash , fiber , tannin , protein content and cook ability of seeds

b- Effect of N P – fertilization on legumes :

1-Vegetative growth :

Many researchers observed that N application to leguminous plants increased plant growth, dry matter content (Hassan *et al*. , 1993 ; Abou El-Salehein *et al* .,1995; El-Beheidi *et al*., 1996 and Fekry *et al* .,2003).

Akhtar *et al*. (2003) found that vine length , number of pods/plant , pod length, number of grains pod and green pod yield were significantly affected by the levels of P_2O_5 . Number of grains pod and green pod yield were maximum at the highest dose of P_2O_5 (69 kg ha^{-1}). However , vine length , number of pods/ $plant^{-1}$ and pod length increased up to the level of 46 kg P_2O_5 ha^{-1} . Application of K_2O to the crop up to the dose of 100 kg ha^{-1} had significant positive effect on all the parameters studied, beyond this dose further application of K_2O was ineffective as combined effect of P_2O_5 and K_2O is concerned , it significantly influenced the pod length and green pod yield . Maximum pod length was attained when 69 or 46 kg P_2O_5 ha^{-1} was applied in combination with 100 or 150 kg K_2O_5 ha^{-1}. Green pod yield was maximum at 69 kg P_2O_5 ha-1 ×100 or 150 kg K_2O ha^{-1} .

Nosheen and Shafique (2006) reported that a significant effect of phosphorus application was observed on the growth and nodulation of inoculated chickpea (*Cicer aritinum L*.)

Erman *et al*.(2009) showed that phosphorus application on pea had significant effect on the plant height , number of branches , root and shoot dry weight .

Ashraf *et al*. (2011) showed that growth parameters of pea like main stem length (cm), number of leaves per plant, leaf area (cm^2), number of pods per plant were increased with different combinations of irrigation, potassium and phosphorus fertilizers

Fatima *et al*.(2013) observed that the effect of different nitrogen and phosphorus levels on growth and yield of lentil (*Lens culnaris*) was significant and consistent increased at all growth stages up to 45 kg N ha^{-1} where as leaf area index and numbers of branches per plant increased significantly with nitrogen application up to 30 kgN ha^{-1}. The phosphorous application significantly increased growth characters viz , plant height , number of nodules and number of branch / plants and dry matters acumulation up to 50 kg P_2O_5 ha^{-1}.

2- Crop yield and its components:

Many investigators showed that the effect of N application on yield and its components on leguminous plants i.e(Hassan *et al*. 1993**,** and El-Beheidi *et al*.,1996, on peas and Abou El-Hassan *et al*., 1993 , Abou El-Salehein et *al*., 1995 and Fekry *et al*., 2003, on beans. On the other hand , Fekry *et al*. (2003) showed that using the levels of 20 kg N/fed., combined with 31 kg P_2O_5 / fed., produced the highest values of different studied characteristics of bean green pod yield and its components.

Application of recommended dose of K_2O (60 kg ha^{-2}) produced highest yield of pea. It has reported that the application of recommended dose of NPK with trash incorporation recorded higher cane and commercial cane sugar yield , the beneficial effects of integrated use of organic manure and inorganic fertilizers on the soil physic-chemical properties and crop yield have also been observed (Bhalarao *et al* ., 2001).

Achakzai *et al*. (2006) showed that components of pea cultivars (except pod length and protein contents) and most of their response toward applied rate of fertilizers were found highly significant ($p< 0.01$). The maximum fresh pod yield plant (143.54 g) , fresh pod yield ha^{-1} (11908.50) , number of fresh pod plant (20.65) , fresh pod length (9.06 cm), 1000 dry seed weight (240.13 g) and protein content

(223.6 g kg^{-1}) of air dried seeds were recorded in fertilizer dose of 100 + 60 + 40 kg NPK ha^{-1}.

In addition , Erman *et al*. (2009) reported that phosphorus application on pea and significant effect on the plant seed and biomass yields , number of pods and phosphorus content of seed.

Yakubu *et al*. (2010) found that phosphorus application at 20 and 40 kg P ha^{-2} improved the performances of cow pea significantly ($P \leq 0.05$). application of 40 kg P ha^{-2}increased the nodule number by 153%, N content in the plant tissue by 288%, and amount of N fixed by 378% over the control.

Plants at P deficiency and low levels of P supply had lower growth rates , higher ureides and amino acids concentration in nodules and roots than plants grown with high (100 mg P kg^{-1} soil) P. The same trend of P influence was denoted in plants subjected to drought stress (35 %WHC) . (Rotaru ., 2010).

Growth parameters of pea like number of pods per plant, length of pod (cm), number of seeds per pod and reproductive attributes like 1000 seed weight , seed yield / hectare were increased with different combinations of irrigation, potassium and phosphorus fertilizers (Ashraf *et al* ., 2011).

Khanday *et al*.(2012) showed that application of the recommended dose of NPK through fertigation gave significantly higher pod yield of pea than all other interactions.

Fatima *et al*.(2013) recorded that the interaction affect of seed yield between N & P was significant where highest with treatment combination of 30 kg N + 50 kg P_2O_5 ha^{-1} and 45 kg N+ 50 kg P_2O_5 ha^{-1} respectively. proved beneficial for highest seed yield of Lentil under temperate condition .

3- Seeds yield and chemical composition:

Many investigators showed that N application increased leguminous plants yield and its quality (Hassan *et al*., 1993, and El-Beheidi *et al*., 1996, on peas and Abou El-Salehein *et a*l.,1995, and Fekry *et al*.,2003).

Fekry *et al*.(2003) showed that using the levels of 20 kg N/fed .,combined with 31 kg P_2O_5/ fed., produced the highest values of different traits of bean yield and its components.

Amjad *et al* .(2004) reported that nitrogen dose (46 kg N ha^{-1}) was kept constant and applied in two split doses , i.e. half at the time of sowing while the other half was applied before flowering . Seed yield , 1000 seed weight and percentage of large sized seeds (< 6.25 mm in dia.) increased significantly with increasing the level of P_2O_5 . However , time taken to complete 50% germination and electrical conductivity of seed leachates decreased significantly . Application of K_2O to the crop had significant positive influence on seed yield , 1000 seed weight and percentage of large sized seeds up to the dose of 100 kg ha^{-1} . Time taken to complete 50% germination and electrical conductivity of seed leachates were also affected significantly being minimum at the dose of 100 kg K_2O ha^{-1} . Application of P_2O_5 and K_2O to the crop had no significant effect on seed germination percentage .

In addition , Erman *et al* .(2009) reported that phosphorus application on pea had significant effect on the seed and biomass yield , number of pods and phosphorus content of seed.

Niri *et al* .(2010) showed that Nitrogen at the rate of 25 kg/ha increased number of filled pods per plants , grain yield harvest index , percentage application at the rate of 40 kg ha^{-1} increased percentage of both grain nitrogen and protein. To obtain the optimal grain and protein yield in lentil under dry land condition , nitrogen and phosphorous levels with 25 and 40 kg ha^{-1} , respectively could be recommended .

Ashraf *et al* .(2011) reported that Growth parameters of pea i.e. number of seeds per pod and reproductive attributes like 1000 seed weight, seed yield / hectare were increased with different combinations of irrigation , potassium and phosphorus fertilizers .

Materials and Methods :

The experiment of the study was carried out at private farm in Tripoli . The soil and irrigation water samples are analysised in Tables(1 and 2) .

Table (1) : The physical and chemical properties of farm soil in current study.

1-Physical properties	**Values**
Sand %	55
Silt %	25
Clay %	20
Soil texture	Sandy loam
Organic Matter	0.5%
pH	7.6
CEC. (Cation exchangeable capacity)	21.0 meq/100g.soil.
2-Chemical properties	
Ca^{++} meq/L	1.71
Mg^{++} meq/L	1.02
Na^{+} meq/L	3.01
K^{+} meq/L	0.02
HCO_3^{-} meq/L	0.53
Cl^{-} meq/L	4.89
SO_4^{-}meq/L	0.28
Available N ppm	91.0
Available P ppm	108.0
Avilable K ppm	324.0
EC	6.3 dS/m

Table (2):Irrigation water properties analysis.

Item	Values
Total salts(ppm)	1580
Sodium (%)	61
Adj. SAR	6.00
Ca^{++} meq/L	17.00
Mg^{++} meq/L	18.30
Na^{+} meq/L	17.78
K^{+} meq/L	2.02
SO_4^{--} meq/L	4.34
Cl^{-} meq/L	8.18
HCO_3^{-} meq/L	3.10
BO_3^{-} meq/L	3.22

SAR= Adjustment of sodium absorption ratio.

meq/l = ml equivalent per liter.

The temperature and relative humidity of Tripoli zone during period of the study ,Table (3)..

Table (3): The temperature and relative humidity of Tripoli zone during period of the study.

Month	Temperature			Relative Humidity %
	Min.	Max.	Mean	
January	7.6	18.8	12.9	58
February	6.9	18.9	12.4	56
March	12.1	26.1	18.5	44

The land was prepared by deep ploughing, harrowing then leveling and ridging. The land was then divided into plots, each plot area was 6.3 m^2 which were three ridges, 3.0 m length and 70 cm width . seeds were sown on both sides at 15 cm apart within every ridge and spray irrigation system were used.

In Treatments with nitrogen fertilizer, 160 kg N /ha. in the form of ammonium sulphate (20.5 % N). Phosphorus fertilizer, 300 kg calcium superphosphate (16.5 % P_2O_5). Nitrogen and phosphorus fertilizers were broadcasted in the two equal doses at 15 and 30 days after sowing. Also, 200 kg K_2O / ha., in the form of Potassium sulphate (48 % K_2O) was added before sowing .

. Certified seeds of peas (*Pisum sativum* L.) cv. Master-B were obtained from Hort. Res. Inst., Agric. Res. Center, Egypt. Pea seeds were sown on November 25thin growing season and tested before sowing with the test of germination , that the germination percentage was 98 % .

The treatments were as follows :

T1= Control (without any addition)
T2= Rhizobium (1.5 kg /hectare)
T3= Bacillus (1.5 kg / hectare)
T4= N & P fertilizers.
T5= Rhizobium + Bacillus
T6= Rhizobium + N& P fertilizer.
T7= Bacillus + N& P fertilizer.
T8= Rhizobium + Bacillus + N& P fertilizer.

The experimental plots are laid out in a randomized block design (RBD) with three replicates.

Biofertilizers obtained from the General Organization for Agriculture Equalization fund (GOAEF) Ministry of Agriculture, Egypt were mixed with wet seeds by adding Arabic Gum solution before sowing and the treated seeds were directly sown in the same day . The biofertilizers okadein contains a nitrogen fixing bacteria (*Rhizobium leguminosum*), specially for peas and phosphorein contains *Bacillus megatherium* phosphate- dissolving bacteria were used in this experiment.

Data are recording :

1-Vegetative growth :

Sample of five plants from each plot was randomly chosen at 60 days after sowing and data were recorded on the following variables :

1-Plant height (cm) .

2-Number of leaves / plant .

3-Dry weight of leaves / plant (g) .

2-Green pods yield traits:

Fresh pods yield of each plot are harvested 3 times weekly and green pods yield per hectare was calculated .

The data were recorded on the following variables :

1-Average pod weight (g) .

2-Mean number of pods .

3 -Total green pods yield / plant (g) .

4-Total green pods yield / hectare (ton) .

3- Seed traits:

1-Average number of seeds / pod .

2-pod length (cm).

3-pod diameter (cm).

4- Chemical analysis of the seeds:

Protein percentage of seeds was determined by multiply nitrogen content in 6.25 according to A.O.A.C (1980)

Total soluble sugars % was determined colorimeterically using the method described by Dubis *et al*.(1960).

Total soluble solids % (T.S.S%): it was determined with Zeiss hand refractometer .

Ascorbic acid (mg / 100 g. F.W.) was determined by the method described by Mattk John (1970).

Statistical Analysis :

The analysis of data was carried out by Analysis Of Variance (ANOVA) . While , Duncan's Multiple Range test was used to test the differences among means (SAS, 2006).

The model used for traits under study was as follows:

$$\mathbf{Y_{ij} = \mu + Treat_i + e_{ij}}$$

Where:

Y_{ij}: The trait of study;

μ: the overall mean;

$Treat_i$: The effect of i^{th} treatments (I = 1,2,3, …. and 8) and

e_{ij}: Random error particular to the ij^{th} observation and assumed to be independently randomly distributed (o and σ^2).

Also, Pearson correlation coefficient was used to find the direction and strength of relationships among studied traits.

Results and Discussion :

1- vegetative growth:

Means ± SE, Levels of significance and comparison of mean by Duncan's Multiple Range Test for Effects of bio-fertilizers and N& P fertilizers treatments alone or in combination with them on plant height (cm); number of leaves and dry weight of leaves (g) traits are presented in (Table 3 , Figs 1-3) .

Table (4) : Effects of biofertilizers and N&P fertilizers on the growth characters of peas.

Treatments	Plant height (cm)	Number of leaves	Dry weight of leaves (g)
Overall mean	** 60.56 ± 0.52	** 26.19 ± 0.26	** 5.44 ± 0.14
Control	56.13 ± 0.03 h	24.46 ± 0.0.5 h	4.61 ± 0.03 h
Rhizobium leguminosum	59.25 ± 0.0.4 f	25.22 ± 0.02 f	4.95 ± 0.02 f
Bacillus megatherium	58.19 ± 0.03 g	24.60 ± 0.02 g	4.78 ± 0.01 g
N& P fertilizers	61.27 ± 0.04 d	26.31 ± 0.02 d	5.41 ± 0.02 d
Rhizobium+ *Bacillus*	60.16 ± 0.03 e	26.15 ± 0.02 e	5.16 ± 0.02 e
Rhizobium+N&P fertilizers	63.12± 0.06 b	27.45 ± 0.01 b	6.09 ± 0.06 b
Bacillus+ N&P fertilizers	62.15 ± 0.02 c	27.13 ± 0.01 c	5.73 ± 0.01 c
Rhizobium +*Bacillus* + N& P fertilizers	64.23 ± 0.05 a	28.20 ± 0.04 a	6.81 ± 0.04 a

**** Highly significant at level 1% ($p \leq 0.01$)**
Means with the same letter are not significantly different.

A highly significant effect ($p \leq 0.01$) for treatments on previous traits was observed. The overall means of plant height ; number of leaves and dry weight of leaves averaged 60.56 ± 0.52; 26.19 ± 0.26 and 5.44 ± 0.14, respectively.

All treatments produced greater yield as compared by control. The best yield was recorded for (Rhizobium + Bacillus + N& P fertilizers) treatment; the lowest yield (56.13±0.03) was obtained for the control and the other treatments came in between.

On the other hand , the higher values (of yield for the previous parameters were obtained from (N& P fertilizers) treatment (62.15±0.05) whether used alone or in combination with the microbial inoculants (Bio-fertilizer) treatments compared to Bio-fertilizer treatments alone.

This result with that is in accordance to reported by (Rather *et al*., 2010) studied the effect of bio-fertilizers on growth, yield and economics of field pea (*Pisum sativum*). They found the bio-fertilizer treatment (Rizobium + Azotobacter and phosphate solubilizing bacteria) gave highest growth in terms of plant; number of leaves/plant; dry wt. of nodels

The variation in the yield traits according to treatments in present study could be attributed to microbial processes like biological nitrogen fixation and phosphate solubilization, which could supplement the nutrient requirements of crops. The contributions of these microbial processes are enhanced by introducing efficient microbes in the Rhizosphere. For example, symbiotic nitrogen fixation rates could be markedly increased by introducing highly efficient, competitive and persistent strains of Rhizobia. Besides, solubilizing the native phosphorus sources in the soil, phosphate solubilizing microorganisms could increase the effectiveness of mineral p fertilization. Also, inoculation of seeds or soil with efficient nitrogen fixing and phosphate solubilizing microorganisms change the rhizosphere population, consequently affecting plant growth.

The significant effect of Rhizobium + N&P fertilizer on the growth traits of pea in current study is similar with obtained by Taiwo *et al*. (1999) They found that there is a high requirement of P by legumes as this element has a pronounced effect on root development , straw length , crop quality and yield of crop. Also, Fatima *et al*. (2006) reported that adequate supply of P with *Rhizobium* strain (mixture inoculation) plays an important role in physiological and developmental process in

plant life and the favorable effect of this nutrient might accelerate the plant growth, which ultimately resulted in increased yield of

the crop. Requirement of P by legumes as this element has a pronounced effect on root development , straw length , crop quality and yield of crop. The efficiency of *Rhizobium* strain is influenced by the availability of P in the soil as it was directly involved in the nutrition of legume and helps to improve the yield of the crop.

Okadein can be defined as preparations containing live cells of *Rhizobium leguminosum* strain of nitrogen fixing , which can be easily assimilated by plants, concerning the significant effect of Rhizobium + Bacillus in this study , These results are in agreement with findings reported by Subba Rao (1984) and increasing soil fertility , N- fixation and hence plant growth (Pacovsky *et al* ., 1991), while phosphorein provide and dissolves P in the soil which containing live cells of phosphate – solubilizing bacteria , *Bacillus megatherium* var. phosphaticum (Pacovsky and Fuller 1986). Moreover, N and P are important nutrients for plant growth (Mengel and Kirkby , 1982).

Regarding the role of N and P in increasing plant growth , this result is in accordance with the result of Bakry et al .(1984) they noticed that where N increased the efficiency of the plant in building metabolites and phosphorus plays indispensable role in the enzyme system necessary for the energy transform in photosynthesis and respiration (Edmond *et al* ., 1981).

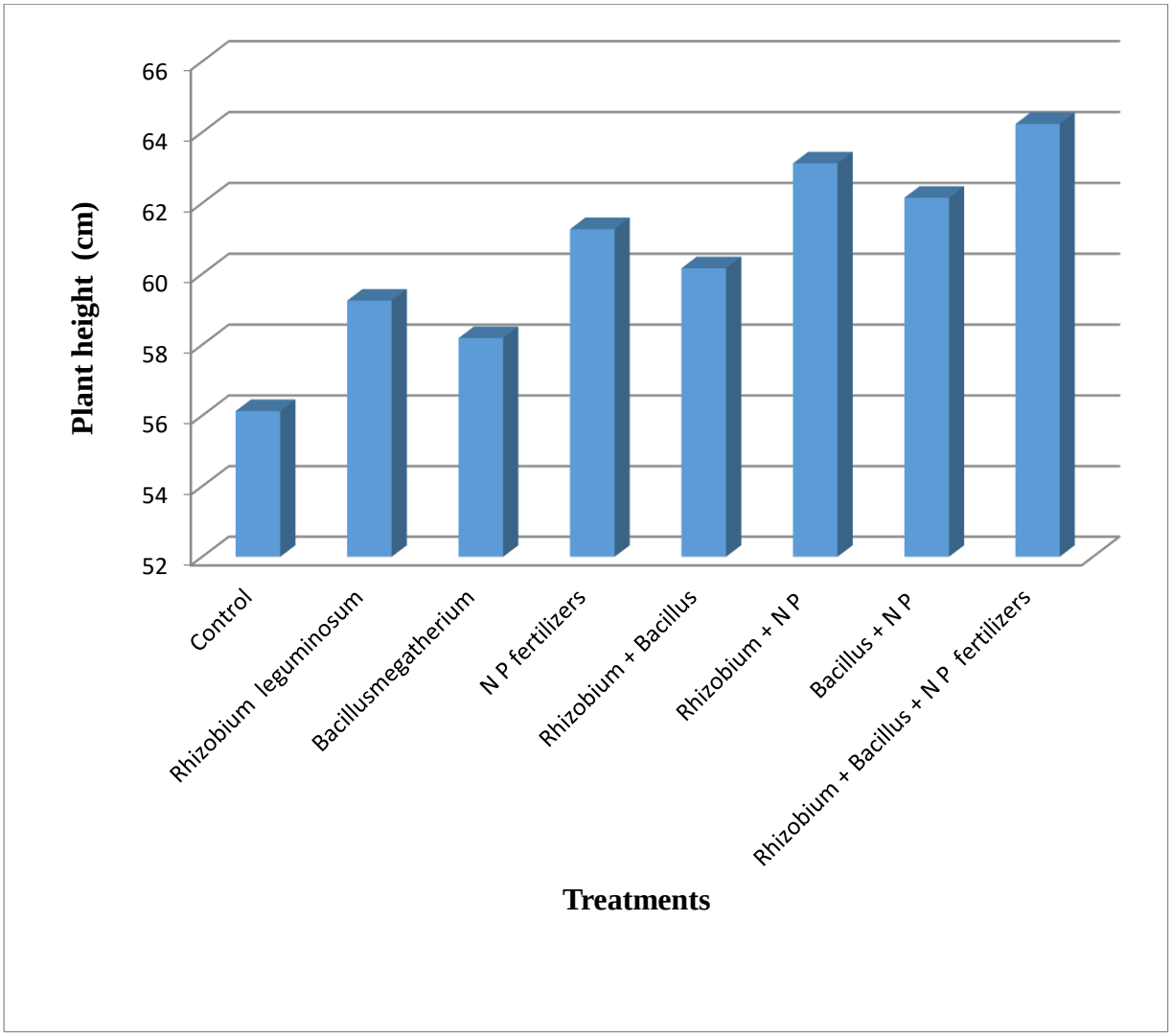

Fig (1) : Effect of bio-fertilizers and N P fertilizers on plant height.

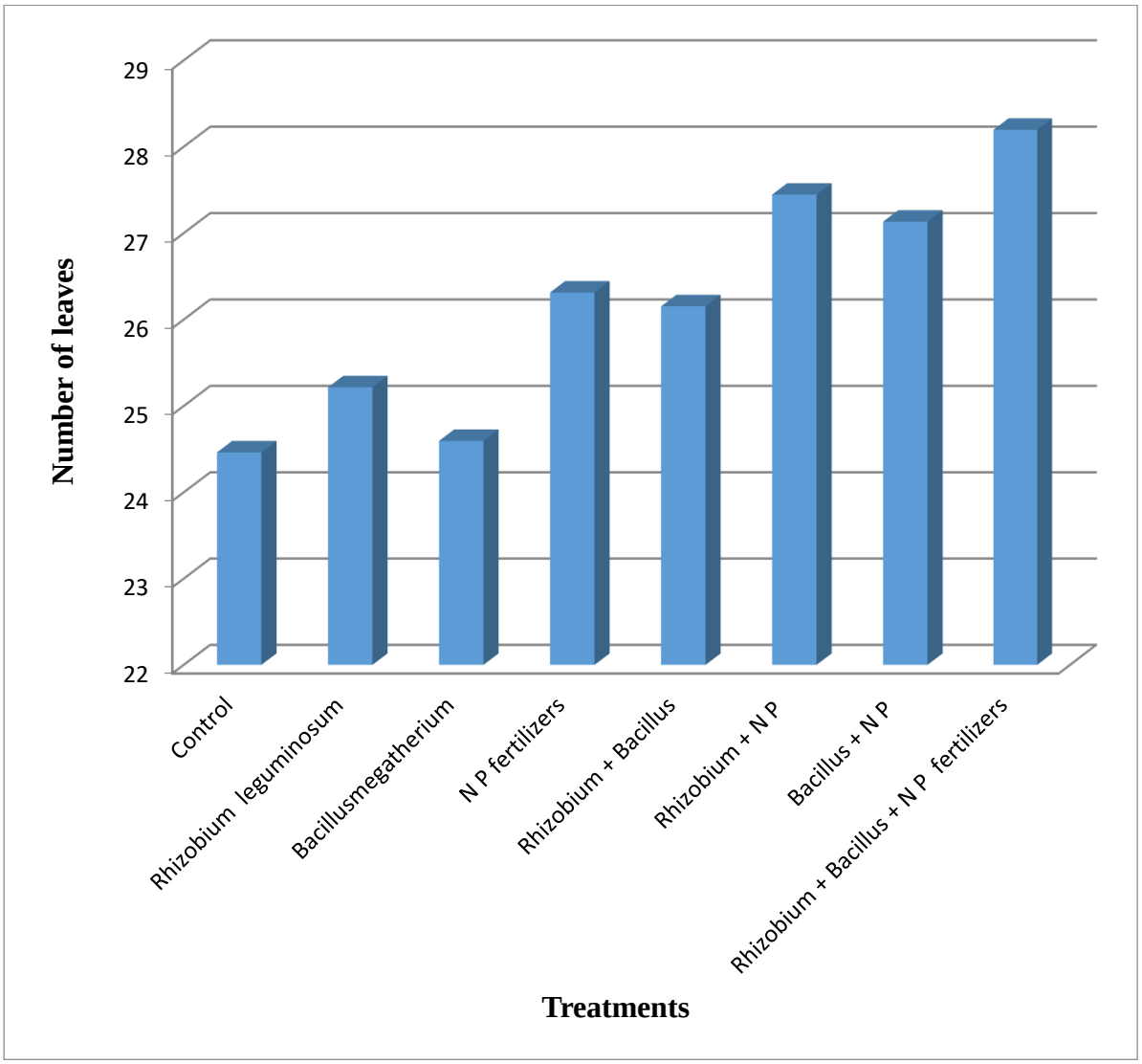

Fig.(2): Effect of bio-fertilizer and N P fertilizers on number of leaves.

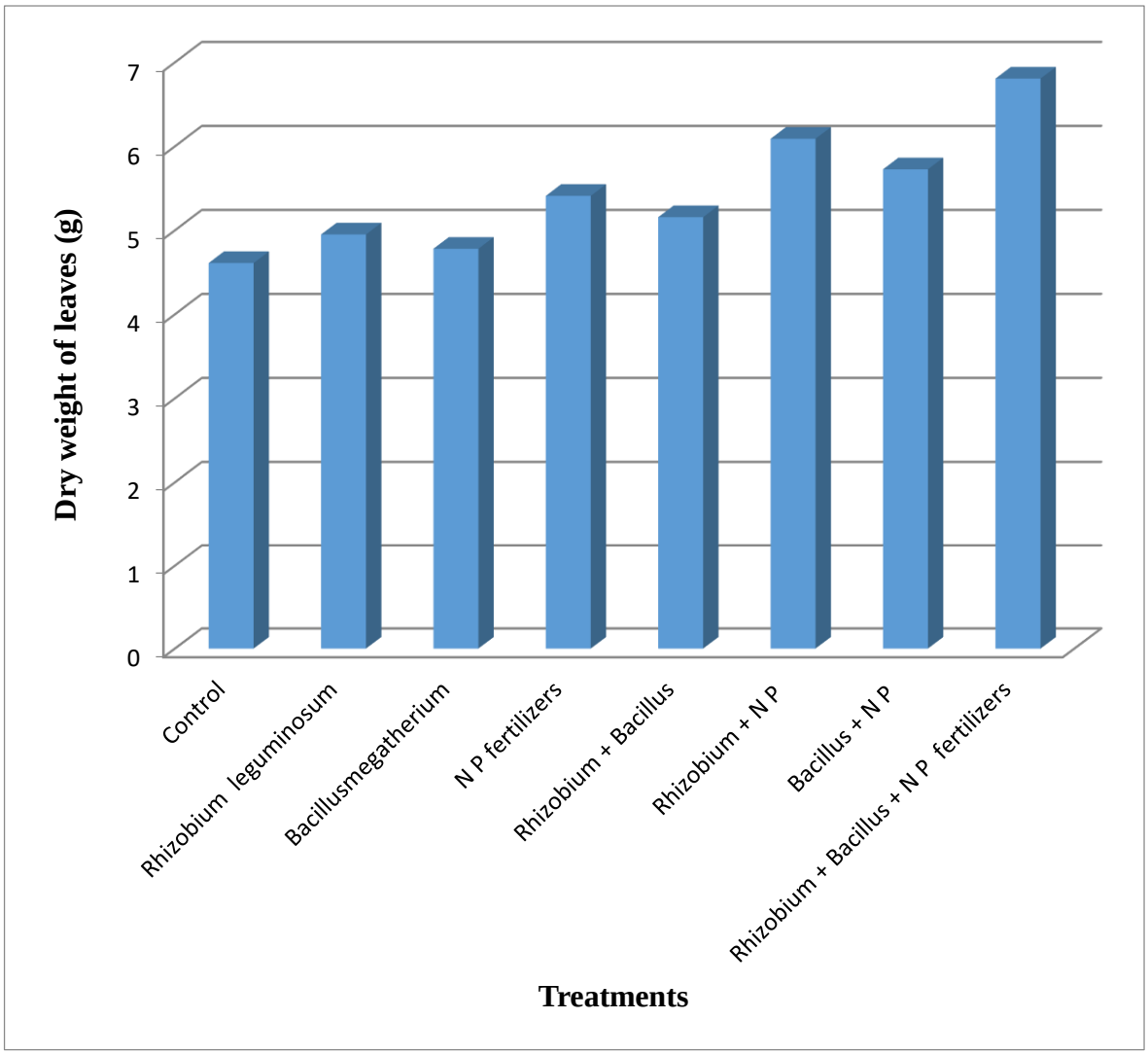

Fig.(3): Effect of bio-fertilizer and N P fertilizers on Dry weight of leaves (g) .

2- Green pod yield and its components :

Means ± SE, Levels of significance and comparison of mean by Duncan's Multiple Range Test for Effects of bio-fertilizers and N& P fertilizers treatments alone or in combination with them on number of pods/plant, average pod weight (g), total green pod yield/ plant(g) and total green pod yield/hectare (ton) . (Table (4) Figures 4-7).

Table (5) : Effects of Bio-fertilizers and N& P fertilizers on the pod yield and its components.

Treatments	Number of pods/plant	Average pod weight (g)	Total green pod yield/ plant(g)	Total green pod yield/hectare (ton)
Overall mean	** 14.27± 0.22	** 5.07 ± 0.05	** 72.69 ± 1.77	** 19.60 ± 0.34
Control	12.80± 0.04 h	4.74± 0.02 h	61.00± 0.22 h	17.95± 0.02 h
Rhizobium leguminosum	13.42± 0.01 f	4.94± 0.03 f	66.28± 0.30 f	18.40± 0.02 f
Bacillus megatherium	13.21± 0.02 g	4.83±0.03 g	63.82± 0.24 g	18.21± 0.02 g
N& P fertilizers	14.25± 0.01 d	5.09± 0.01 d	72.52± 0.15 d	19.24 ± 0.02 d
Rhizobium+ *Bacillus*	14.10± 0.02 e	5.02± 0.01 e	70.83± 0.12 e	18.63± 0.02 e
Rhizobium+N&P fertiliz.	15.21± 0.02 b	5.34± 0.02 b	81.22± 0.41 b	21.25± 0.11 b
Bacillus+ N&P fertiliz.	15.08± 0.01 c	5.22± 0.01 c	78.65± 0.14 c	20.20± 0.01 c
Rhizobium +*Bacillus* + N& P fertilizers	16.09± 0.01 a	5.42± 0.01 a	87.17± 0.23 a	22.93± 0.03 a

** Highly significant at level 1% ($p \leq 0.01$)

Means with the same letter are not significantly different.

The green pod yield and its components was a highly significantly different ($p \leq 0.01$) among the treatments.

The overall means of number of pods/plant, average pod weight (g), total green pod yield/ plant(g) and total green pod yield/hectare (ton) were 14.27± 0.22, 5.07 ± 0.05, 72.69 ± 1.77 and 19.60 ± 0.34 , respectively.

All treatments gave higher production over control. The best yield was recorded for (Rhizobium + Bacillus + N& P fertilizers) treatment (87.17±0.23), the lowest yield was found for the control (61.00± 0.22) and the other treatment came in between. On the other hand , the greater values of yield for the previous traits were found from (N&P fertilizers) treatment whether used alone or in combination with the microbial inoculants (Bio-fertilizer) treatments compared to Bio-fertilizer treatments alone.

This result have the same trend with reported by Parsad *et al*. (1989) they found that the highest green pod yield was obtained with combination of 120 kg $P_2O_5ha^{-1}$ and Rhizobium inoculation. However, Mishra (1999) reported that pod yield increased by added 60 kg $P_2O_5ha^{-1}$ in cowpea crop.

As yield per hectare was calculated from the yield per plant by multiplying with number of plants per hectare , therefore the results followed the same pattern of significance and presented the same trend as green pod yield per plant.

The increase in total green pods yield could be due directly to the increase in both number of pods and mean pod weight which in turn reflected on total yield per plant and per hectare. In addition , nitrogen and phosphorus fertilizers might be attributed to their roles in metabolites synthesized by the plant and promotes increasing the yield of pods , its quality and nutritive value of seeds (Bidwell,1979).

Regarding the role of N and P in increasing plant growth , where N increased the efficiency of the plant in building metabolites this result is in accordance with the result of (Bakry *et al* ., 1984) and phosphorus plays indispensable role in the enzyme system necessary for the energy transform in photosynthesis and respiration (Edmond *et al* ., 1981).

Numbers of filled pods are main components of seed yield that are influenced by the amount of fertilizer placed (Toshij *et al*.,1995).

Regarding the effect of P and N in increasing pod yield and its components , These results in line with those found by (Saini and Thakur, 1996; and Verma *et al*., 1997) they reported that application of nitrogen and phosphorus to pea crop usually results in increased green pod and seed yield.

Also , Patel *et al*. (1998) they reported that significantly increased plant height, number of branches, leaves per plant, number of pods per plant, grain per pod and pod yield were observed when added mixed of NPK fertilizers for the soil.

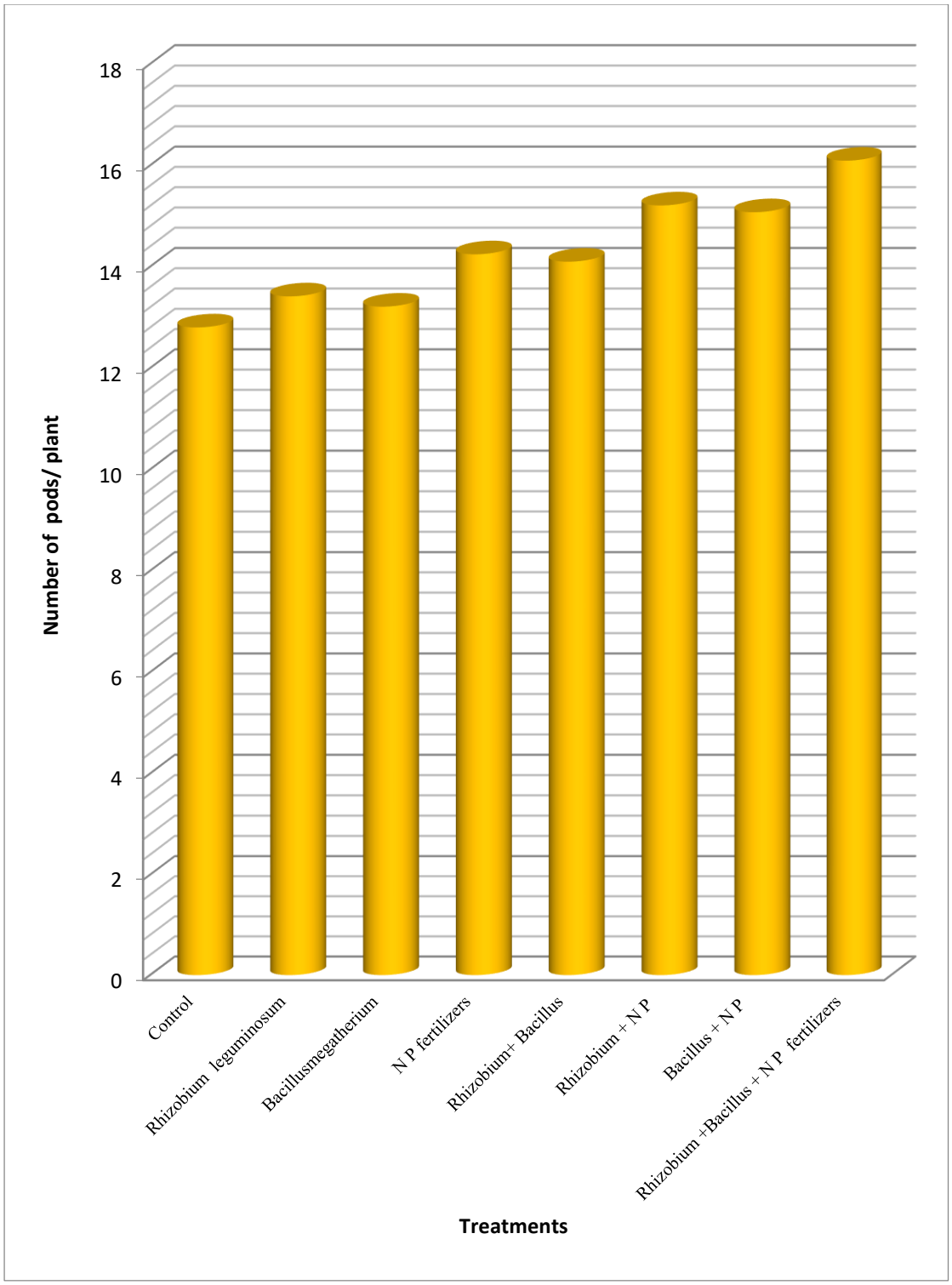

Fig.(4): Effect of bio-fertilizer and N P fertilizers on number of pods/plant .

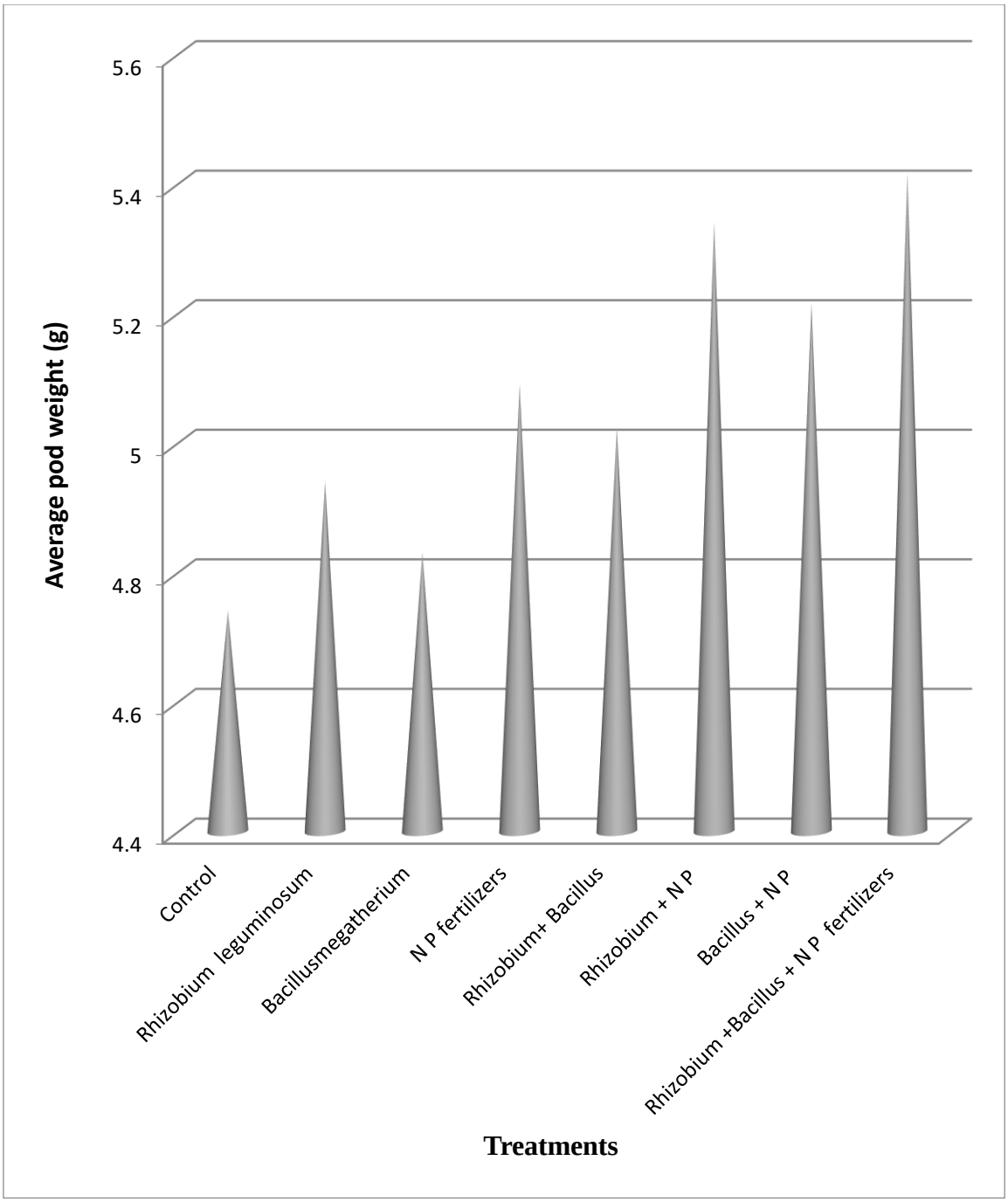

Fig.(5): Effect of bio-fertilizer and N P fertilizers on Average pod weight (g) .

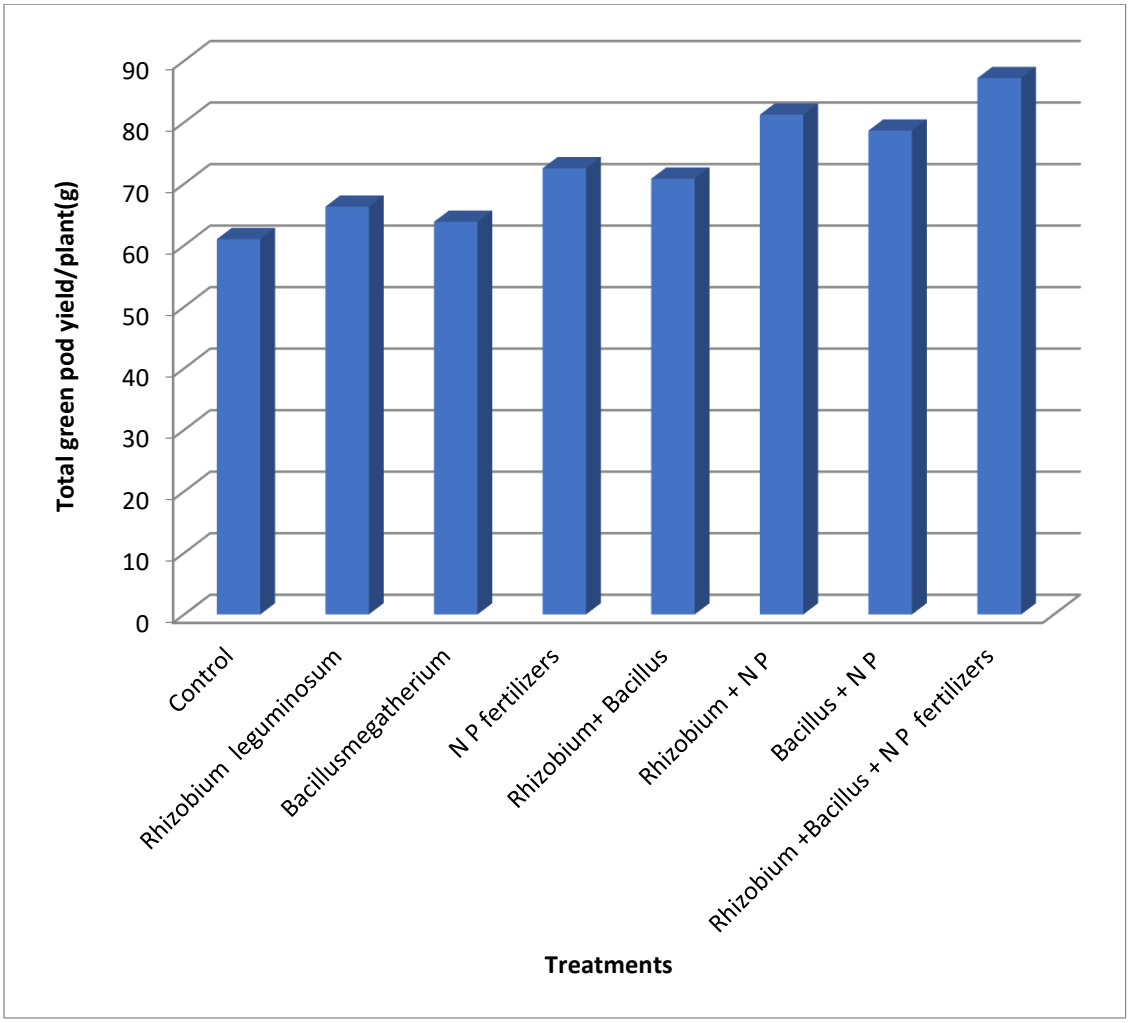

Fig.(6): Effect of bio-fertilizer and N P fertilizers on Total green pod yield/plant (g) .

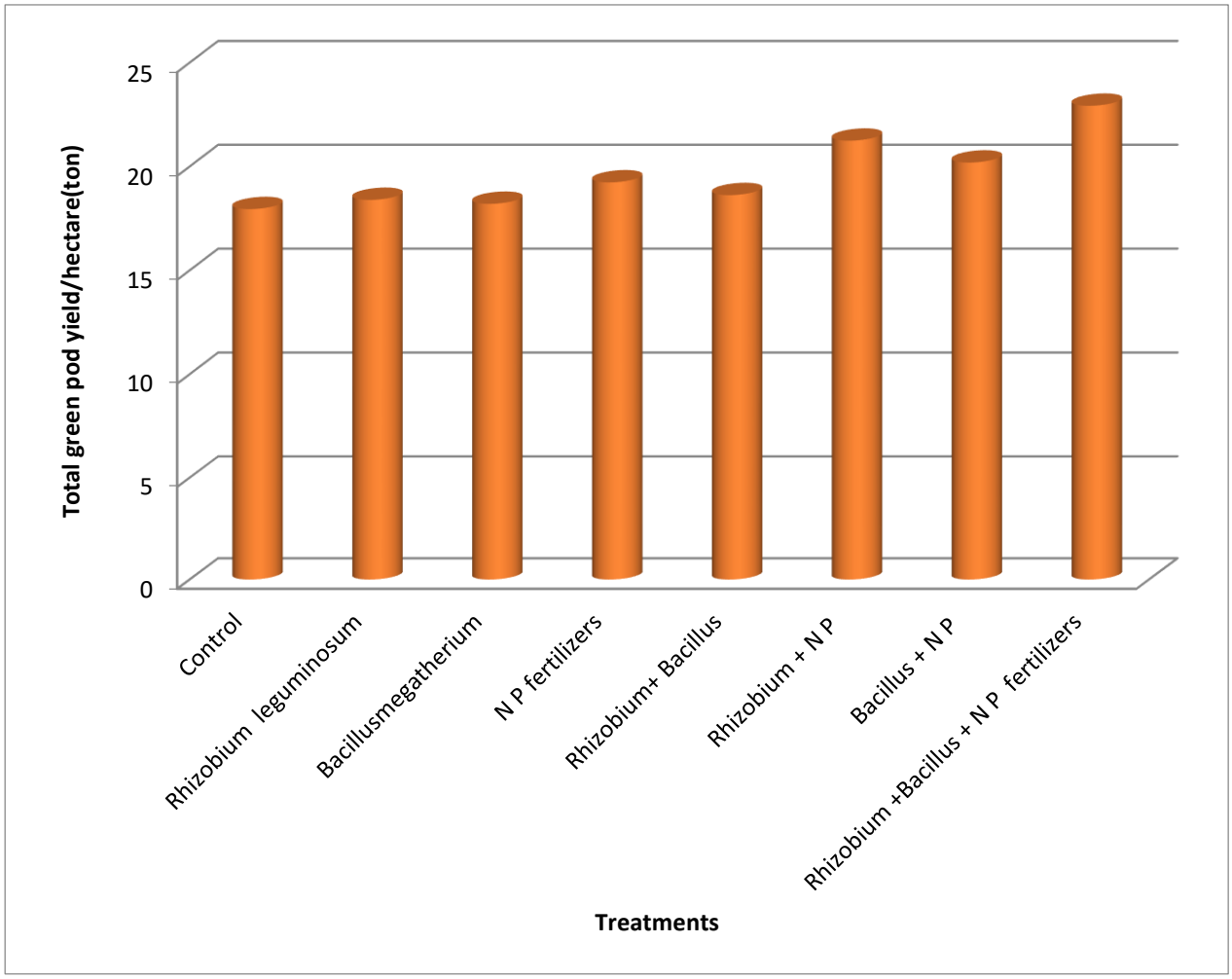

Fig.(7): Effect of bio-fertilizer and N P fertilizers on Total green pod yield /hectare (ton) .

Pod and seed quality :

a- physical pod characters :

Means ± SE, Levels of significance and comparison of mean by Duncan's Multiple Range Test for Effects of bio-fertilizers and N& P fertilizers treatments alone or in combination with them on Number of seeds/pod; Pod length (cm) and Pod diameter (cm) (Table (5) Figs 8-10).

Table (6) : Effects of Bio-fertilizers and N&P fertilizers on pod and seed quality.

Treatments	Number of seeds/pod	Pod length (cm)	Pod diameter (cm)
Overall mean	** 5.27± 0.04	** 6.89 ±0.13	** 1.38±0.03
Control	4.93±0.01 g	6.16±0.02 h	1.14± 0.01 h
Rhizobium leguminosum	5.16±0.02 f	6.42±0.02 f	1.28±0.01 f
Bacillus megatherium	5.12±0.01 f	6.31±0.02 g	1.26±0.01 g
N& P fertilizers	5.32±0.01 d	6.83±0.05 d	1.47± 0.03 d
Rhizobium+ *Bacillus*	5.28±0.05 e	6.66±0.03 e	1.35± 0.04 e
Rhizobium+N&P fertiliz.	5.45±0.02 b	7.43±0.02 b	1.50± 0.01 b
Bacillus+ N&P fertiliz.	5.40 ±0.01 c	7.17±0.03 c	1.49± 0.02 c
Rhizobium +*Bacillus* + N& P fertilizers	5.52±0.01 a	8.12± 0.02 a	1.52± 0.02 a

** Highly significant at level 1% ($p \leq 0.01$)
Means with the same letter are not significantly different.

A highly significant effect ($p \leq 0.01$) for treatments on previous traits was found. The overall means of Number of seeds/pod; Pod length (cm) and Pod diameter (cm)traits averaged 5.27± 0.04; 6.89 ±0.13 and 1.38±0.03, respectively.

All treatments produced greater yield as compared by control. The best yield was recorded for (*Rhizobium* + *Bacillus* + N& P fertilizers) treatment; the lowest yield was obtained for the control and the other treatments came in between. On the other hand , the higher values of yield for the previous parameters were obtained from (N& P fertilizers) treatment whether used alone or in combination with the microbial inoculants (Bio-fertilizer) treatments compared to Bio-fertilizer treatments alone.

Similar results have been reported by previous researchers in pea (Saini and Thakur, 1996; Verma *et al*., 1997; Mishra, 1999 and Gupta *et al*., 2000)

Plant nutrition play an important factor for obtaining higher yield of legume or other crops according to soil fertility which varies greatly and also, the response varies from cultivar to cultivar. Most agricultural soils are generally low in nitrogen (N) and phosphorus (P). These two nutrients are often limiting the crop production . For pulse production, P is the major limiting nutrient followed by N. This is because P not only affects legume growth, but also nodule formation and growth. The phosphate solubilizing microorganisms have capacity to dissolve the insoluble phosphoric compounds present in the soil and also solubilize rock phosphate, bone meal and basic slag.

The field trials done on inoculation of P-solubilizing bacteria in various crops have shown 10-15% increase in crop yield (Mesele and Pant, 2012).

Mishra *et al*. (2010) observed that a highly significant effect of bio-fertilizers in conjunction with inorganic fertilizers on number of grains pod, number and weight of pods plant and grain yield.

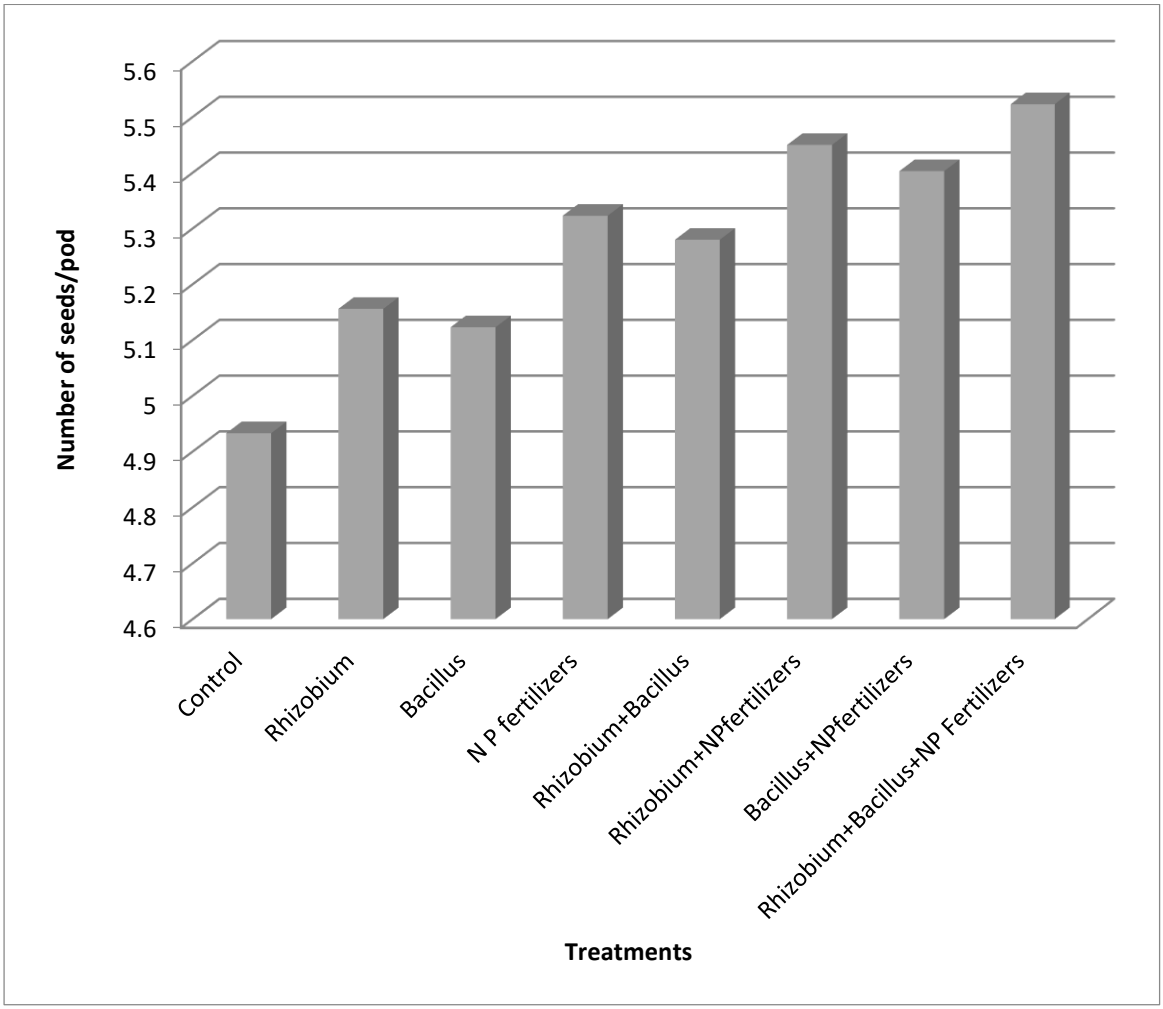

Fig.(8): Effect of bio-fertilizer and N P fertilizers on Number of seeds / pod .

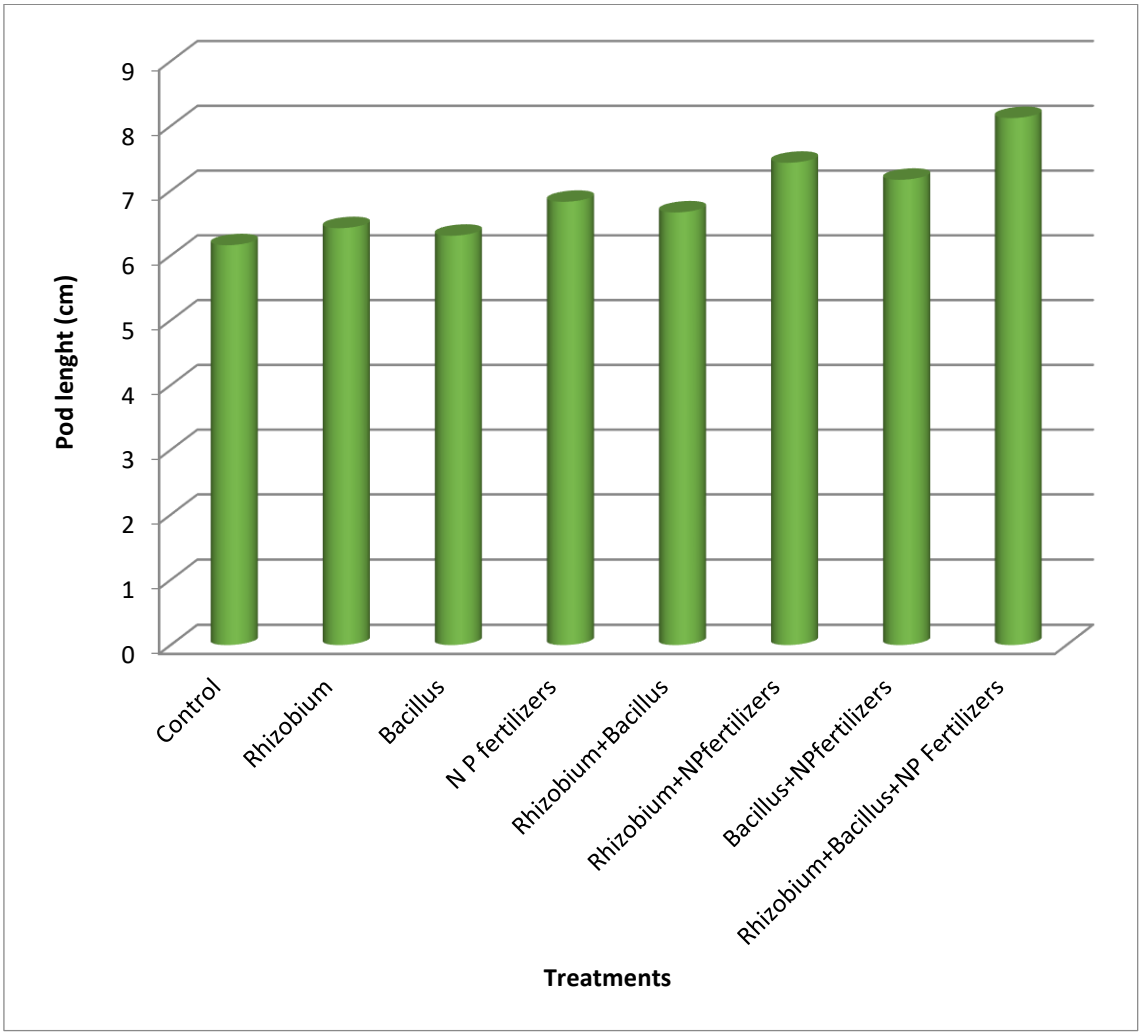

Fig.(9): Effect of bio-fertilizers and N P fertilizers on Pod length (cm) .

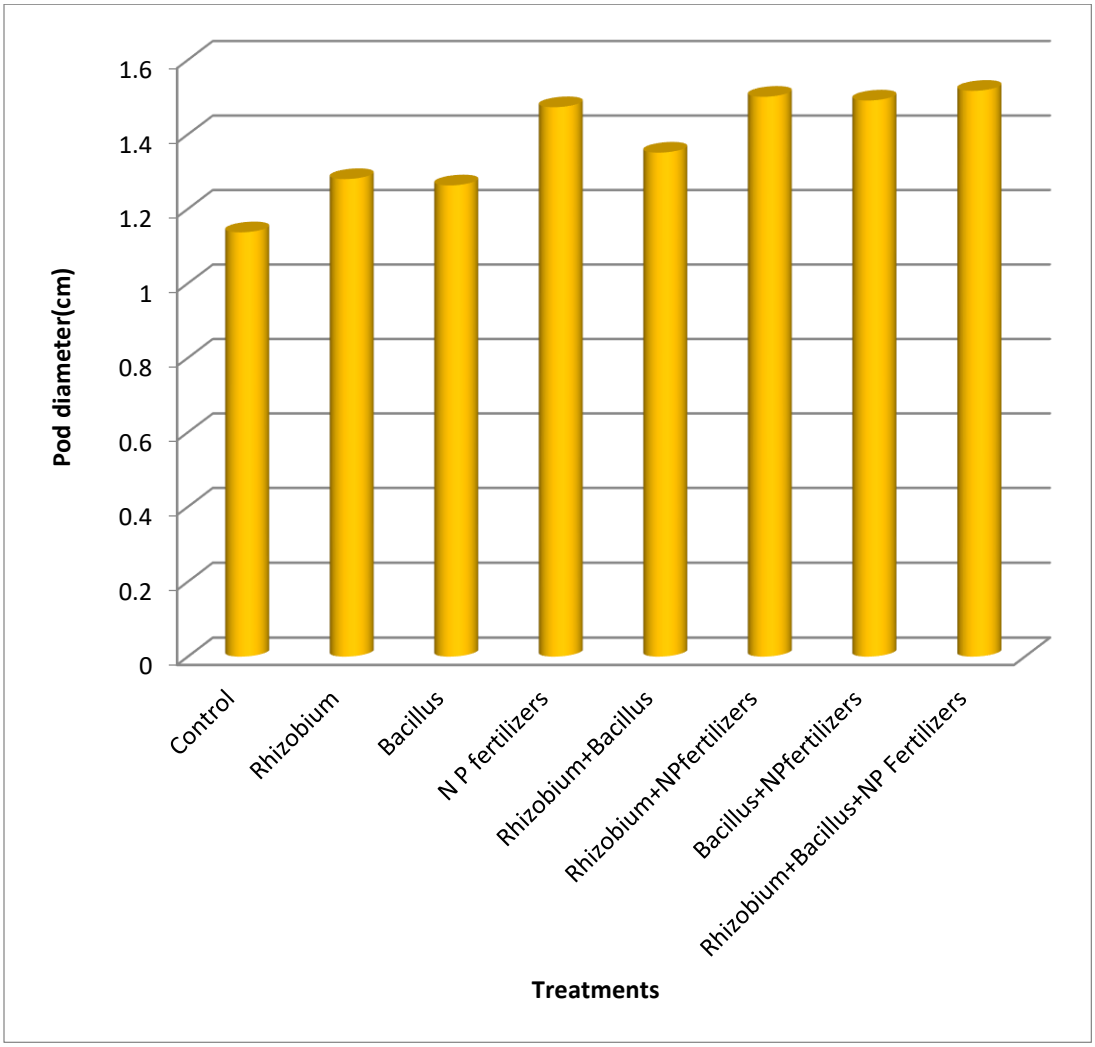

Fig. (10): Effect of bio-fertilizer and N P fertilizers on Pod diameter (cm) .

b- Chemical composition in seed (nutritive value) :

Means ± SE, Levels of significance and comparison of mean by Duncan's Multiple Range Test for Effects of bio-fertilizers and N& P fertilizers treatments alone or in combination with them on T.S.S %, Total soluble sugars %, Ascorbic acid mg/100gm F.W, Nitrogen% and Protein % traits are illustrated in (Table (7) figs 11-15) .

Table (7) : Effect of Biofertilizers and N& P fertilizers on the chemical composition of pea seeds.

Treatments	T.S.S %	Total soluble sugars %	Ascorbic acid mg/100gm F.W	Nitrogen %	Protein %
Overall mean	** 6.00± 0.07	** 3.77 ± 0.05	** 4.47 ± 0.07	** 3.19 ±0.03	** 19.96±0.20
Control	5.77±0.01 bc	3.55±0.01 C	3.92±0.01 h	3.02± 0.02 d	18.88±0.02 D
Rhizobium leguminosum	5.62± 0.31 c	3.77± 0.01 abc	4.26± 0.02 f	3.07± 0.03 cd	19.22±0.02 Cd
Bacillus megatherium	5.59± 0.29 c	3.64±0.01 bc	4.15±0.02 g	3.19±0.14b c	19.93 ±0.08 Bc
N& P fertilizers	6.12±0.02 ab	3.84±0.02 ab	4.54±0.02 d	3.11±0.01 cd	19.43±0.02 Cd
Rhizobium+ *Bacillus*	6.03±0.02 abc	3.51±0.25 C	4.39±0.01 e	3.09±0.01 cd	19.31 ±0.02 Cd
Rhizobium+ N&P fertilizers	6.31±0.01 a	3.94±0.02 ab	4.81±0.01 b	3.32±0.01 b	20.73±0.02 b
Bacillus+ N&P fertilizers	6.20±0.02 ab	3.88±0.02 ab	4.73±0.02 c	3.29±0.01 b	20.57±0.02 b
Rhizobium + *Bacillus* + N& P fertilizers	6.37±0.01 a	4.0±0.02 a	4.93±0.02 a	3.47±0.01 a	21.67±0.01 a

**** Highly significant at level 1% ($p \leq 0.01$)**

Means with the same letter are not significantly different.

The chemical composition of pea seeds was a highly significantly different ($p \leq 0.01$) among the treatments.

The overall means of T.S.S %, Total soluble sugars %, Ascorbic acid mg/100gm F.W, Nitrogen% and Protein % traits were 6.00± 0.07, 3.77 ± 0.05, 4.47 ± 0.07, 3.19 ±0.03 and 19.96±0.20 Protein %, respectively.

The best values for the chemical component of pea seeds were recorded for (Rhizobium + Bacillus + N& P fertilizers) (21.67±0.01) Rhizobium+ N&P fertilizers (20.73 ±0.02) and Bacillus+ N&P fertilizers treatments (20.57±0.02), respectively. On the other hand, the lowest values were found for the control and the other treatments.

The significant effect of fertilizer treatments on chemical composition of seeds in current study ,This result is supported by Pacovsky et al.,(1991) they noticed that Okadein which contained nitrogen fixation bacteria ; Rhizobium , plays an active part in atmospheric nitrogen fixation and absorption of nutrients , i. e. N and P as well as, cell formation and active translocation , and the increase in protein content due to biological fertilizers was reported by Elsheikh (2001).

The moisture content of legume seeds was found to be affected by the relative humidity of surrounding atmosphere at the time of harvest and during storage (Elsayed, 1994).

Phosphorein which contained P–dissolving bacteria; *Bacillus megatherium* var. phosphatica was found to have the ability to dissolve soil complex inorganic phosphate and to supply the grow plants with N and P macro elements and phytohormones which could increase the dry matter accumulation and in turn the N concentration

Also, The nitrogen fertilizer can increase phosphate absorption and also phosphorous led to increasing in protein percentage of grain (Niri *et al*., 2010).

Similar findings have been reported by Abou El- Salehein *et al* **.,** 1994 who used Rhizobium on pea , Elsoni and Osman , 2011 and Rughein and Rugheim and Abdelgani (2011) used Bacillus megatherium on pigeon pea and faba bean , respectively and Fekry *et al*., (2003) who used N on pea and Erman *et al* ., (2009) used P on pea .

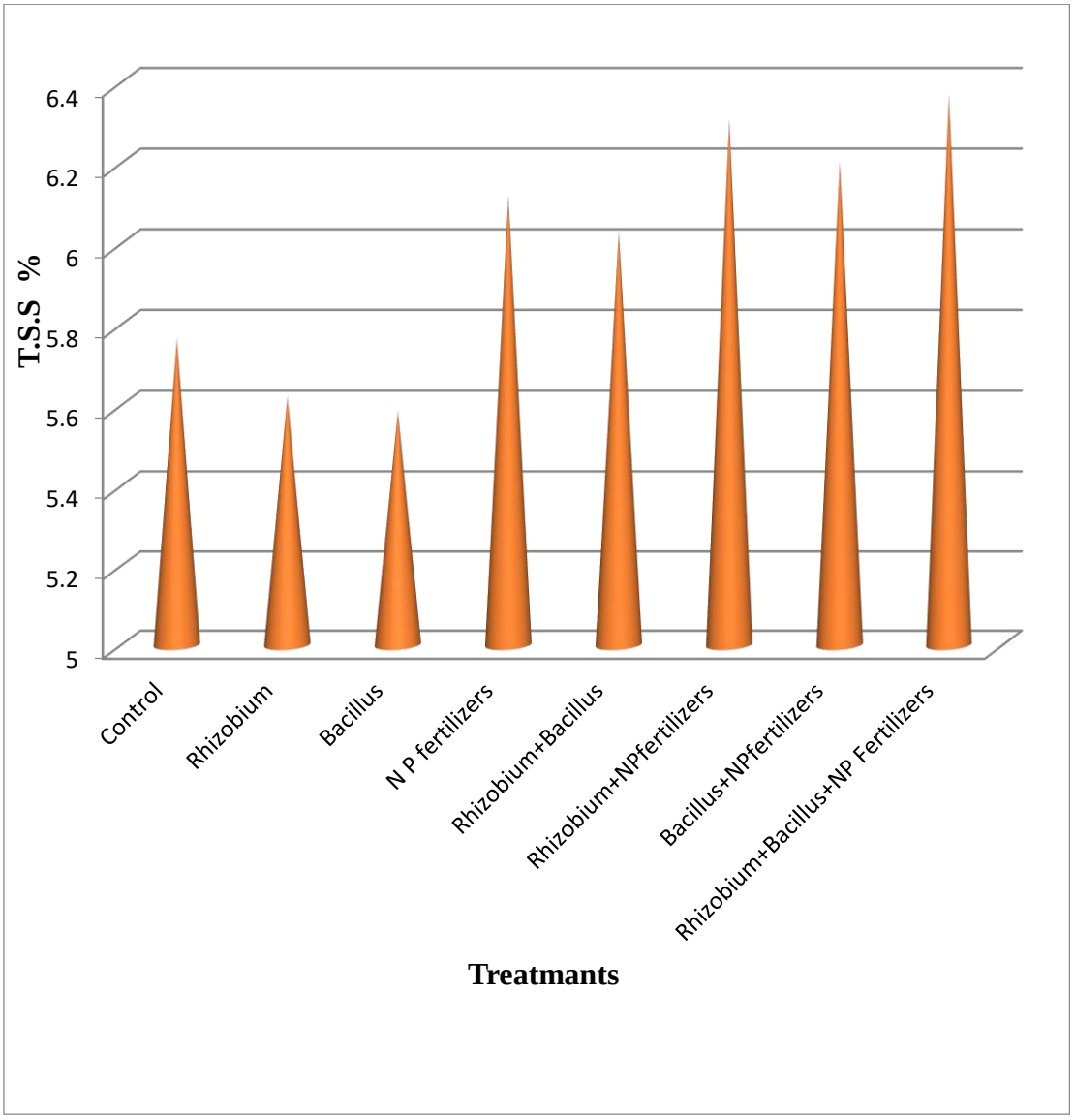

Fig.(11) : Effect of bio-fertilizer and N P fertilizers on T.S.S. % .

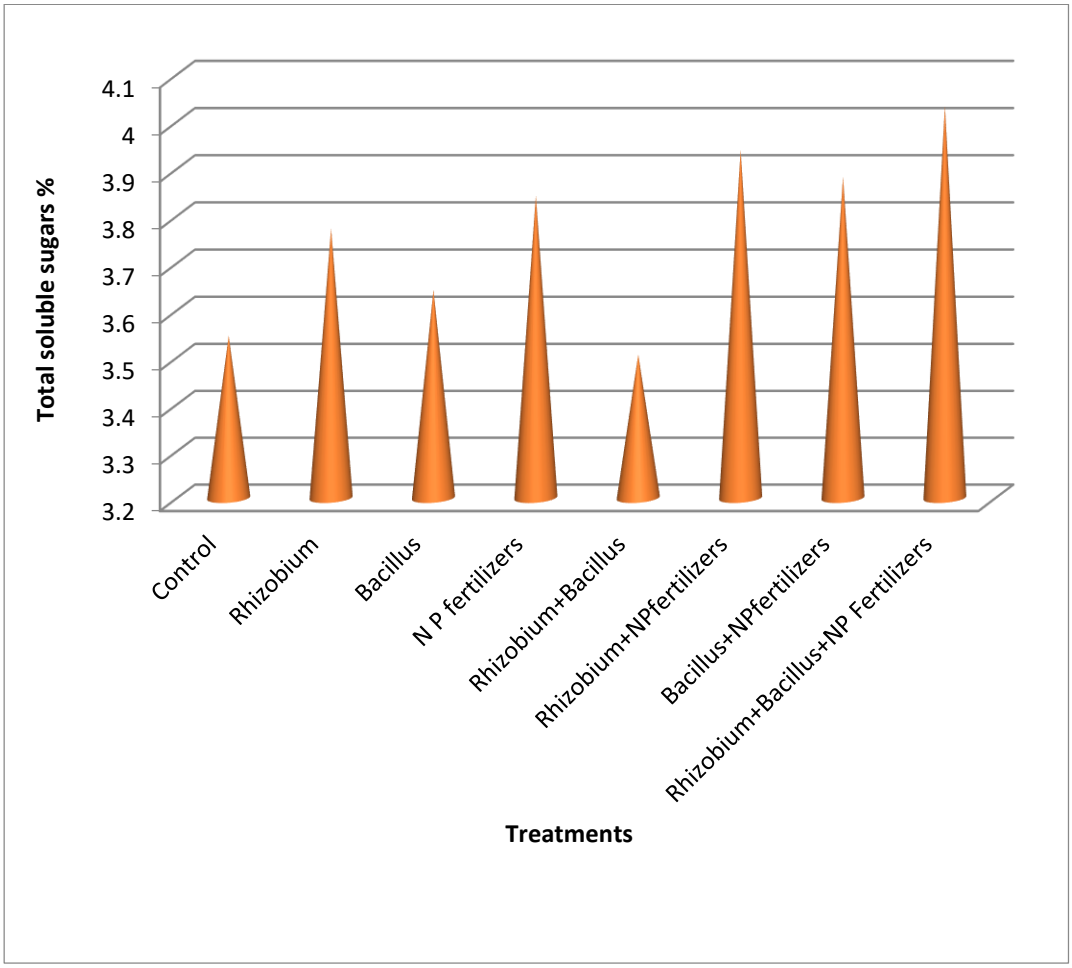

Fig.(12) : Effect of bio-fertilizer and N& P fertilizers on Total soluble sugars % .

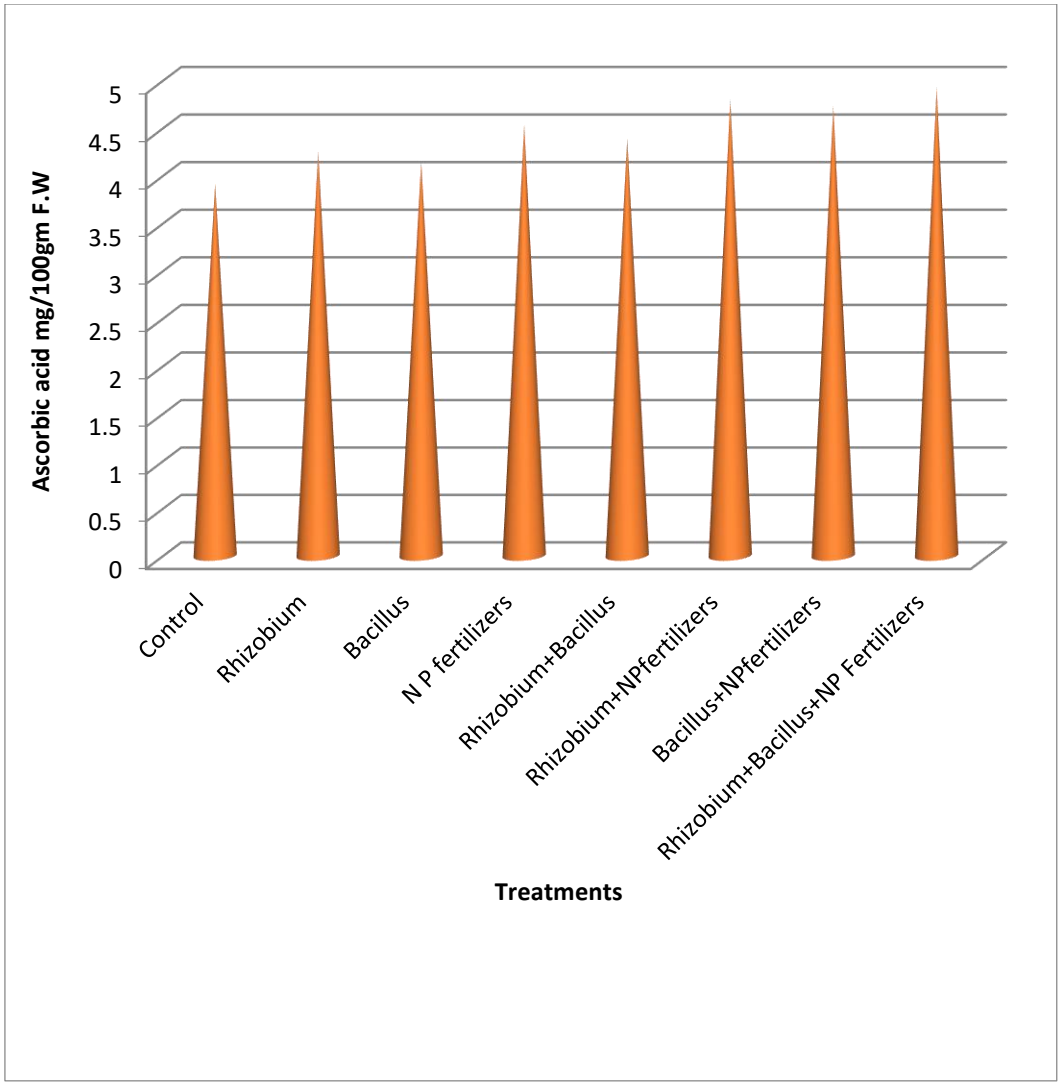

Fig.(13) : Effect of bio-fertilizer and N& P fertilizers on Ascorbic acid mg/100 gm F.W .

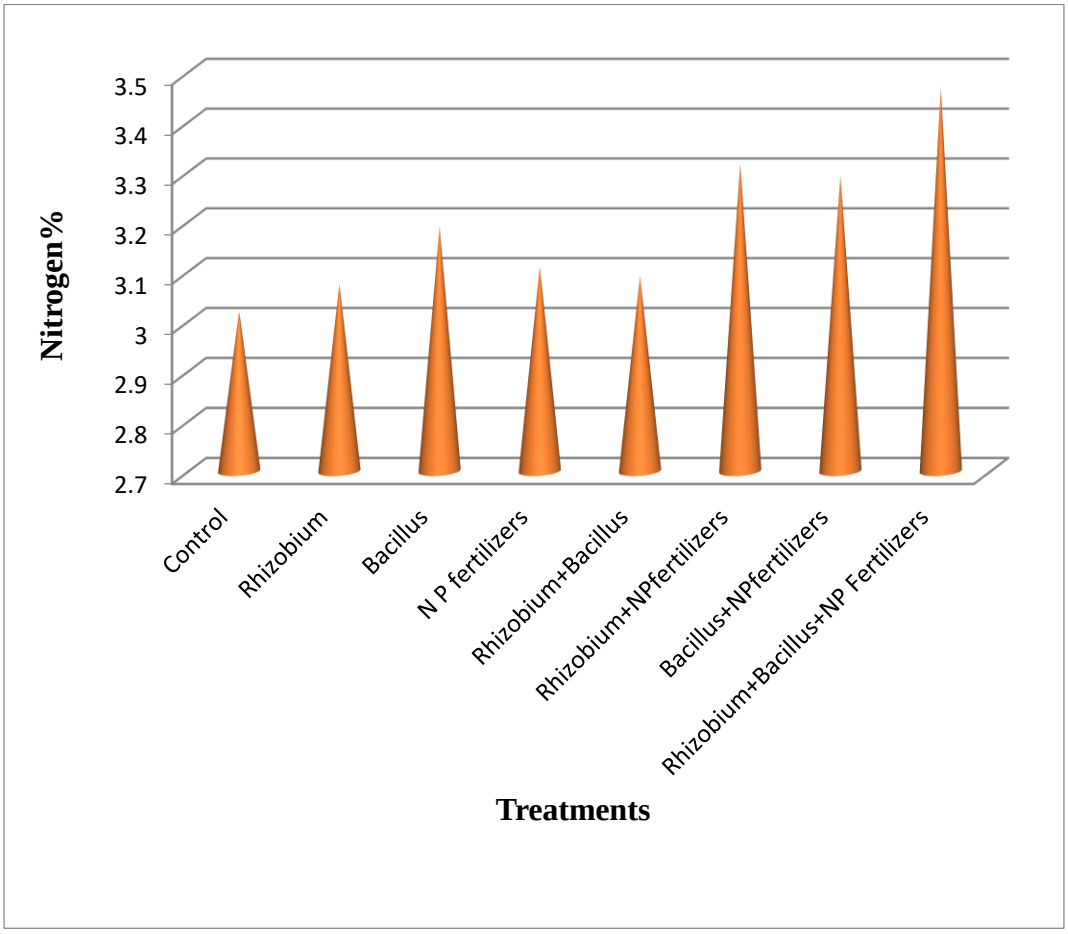

Fig.(14) : Effect of bio-fertilizer and N& P fertilizers on Nitrogen % .

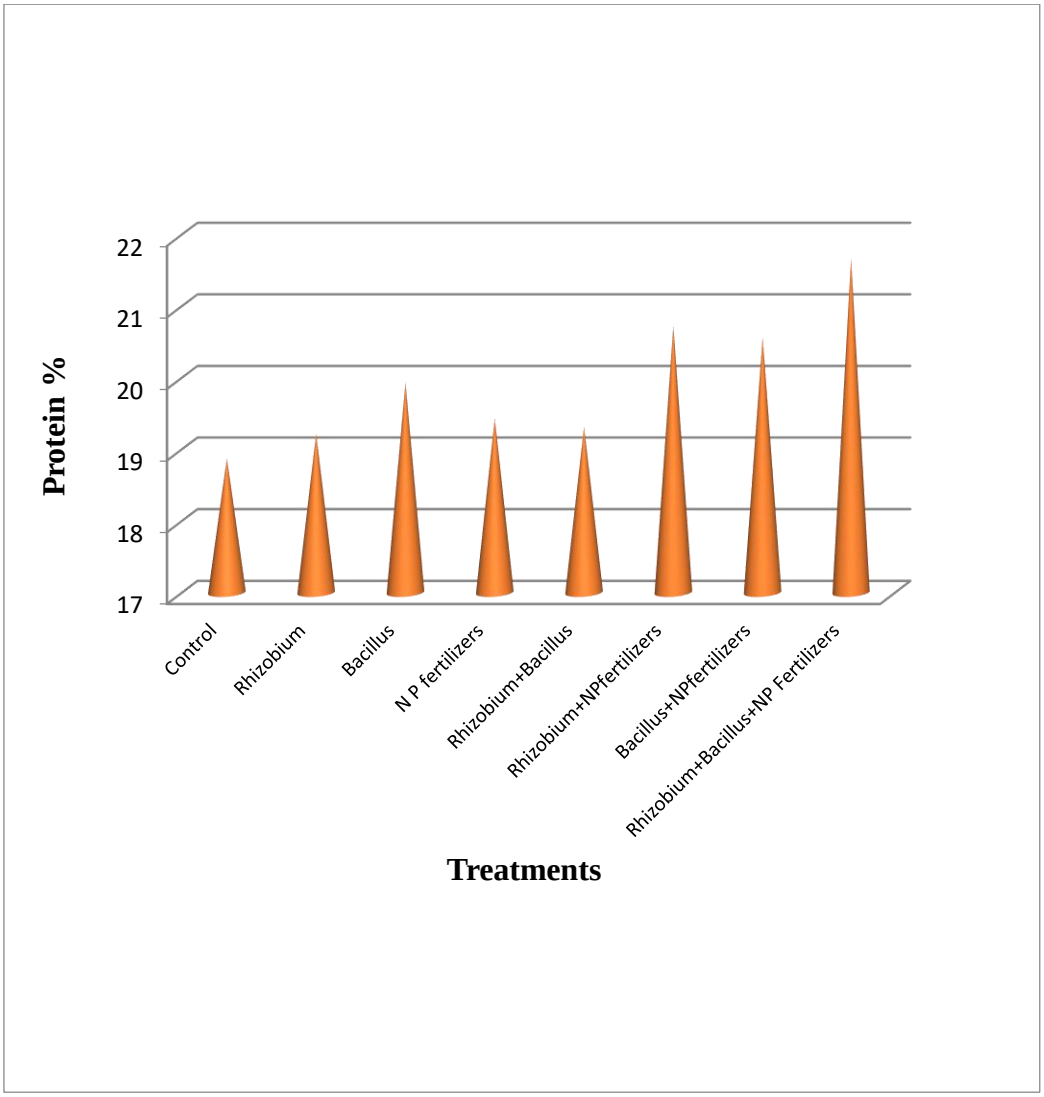

Fig.(15) : Effect of bio-fertilizer and N& P fertilizers on Protein % .

Simple correlation among characteristics (Pearson correlation coefficient) :

The correlation coefficient among different variables are presented in Table (7).

A highly significant positive correlation at level 1% (P≤ 0.01) was observed among different variables of pea. The highest value was 0.99 between total green pod/plant and number of pod /plant, while the lowest value was 0.75 between Number of seeds/pod and protein percentage and the other values came in between.

Table (8) : The correlation coefficient(r) among different variables.

Variable	1	2	3	4	5	6
1- Number of pod / plant	1.00					
2- Average pod weight (g)	0.98**	1.00				
3-Total green pod /plant	0.99**	0.98**	1.00			
4-Number of seeds/ pod	0.96**	0.96**	0.95**	1.00		
5-Pod length (cm)	0.98**	0.96**	0.98**	0.91**	1.00	
6-Protein %	0.80**	0.76**	0.91**	0.75**	0.82**	1.00

**** Highly significant at 1% level (P≤0.01) .**

The obtained values are positively correlated and highly significant in most of studied characters of peas plant, i.e., number of pod / plant , Average pod weight (g) , number of seeds / pod , protein percentage , This result was a similar with found by Khan *et al*. (1983); Kazmi *et al*.(2002); Achakzai & Kayani (2004) and Achakzai and Bungulzai (2006).

Therefore, the information of these relationships is useful and can be used when we put suitable selection programme for the improvement of yield of pea.

Recommendation

Using biofertilizers , i.e. , Okadein and phosphorein , beside the application of N & P fertilizers produced higher yield characters as compared to control , decrease the quantity of chemical fertilizers and the environmental pollution. As well as , save the costs production due to the use of less quantity of chemical fertilizers .

It was concluded from current study that for obtaining maximum grain yield of pea, seed should be inoculated with Rhizobium +Bacillus in combination with N& P fertilizers.

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الملخص

بمنطقة طريق المطار 15 كيلو متر غرب طرابلس) (أجريت تجربة حقلية في مزرعة خاصة بطرابلس لدراسة تأثير الأسمدة الحيوية (العقدين والفوسفورين) (1.5 كجم لكل منهما على حدة) والتسميد النيتروجيني الفوسفاتي (100 كجم ن/ هكتار ، 20.5% ن و 300 كجم سوبرفوسفات 16.5 %) على صفات النمو ، محصول القرون الخضراء ومكوناتها والتحليل الكيميائى لبذور البازلاء صنف ماستر- ب.

أظهرت النتائج أن هناك تأثير عالى المعنوية عند مستوى 1% للمعاملات على جميع الصفات تحت الدراسة ، وأعطت المعاملات أنتاج أعلى فى جميع الصفات مقارنة بالمعاملة الضابطة. وكانت أفضل المعاملات (64.23) المعاملة (رايزوبيوم + باسيللس + السماد النيتروجيني الفوسفاتي) واقلهم المعاملة الضابطة (56.13) ، وجاءت باقى المعاملات وسط بينهما.

بلغ المتوسط العام للصفات الخضرية طول النبات ، عدد الأوراق ، الوزن الجاف للأوراق

± 0.14 على التوالي . كما بلغ المتوسط العام لعدد القرون/ 5.44 ±0.26 و 26.19 ±0.52 ، 60.56; للنبات ، متوسط وزن القرون ، وكلاً من إجمالي محصول القرون الخضراء/ للنبات و للهكتار و عدد البذور ± 5.07 14.27 ± 0.22 فى القرن ، بالإضافة الى عدد البذور لكل قرن وطول القرن وقطر القرن ±0.03 على 1.38 ±0.13 و 6.89 و 0.04 ± 0.345.27 و± 19.60, ±1.77 , 2.69 0.057 التوالى.

±0.07 و 4.47و0.05 ± 3.77 ±0.07 و6.00 كما بلغ المتوسط العام للتحليل الكيميائى للبذور لكل من المواد الصلبة الكلية ، السكريات الذائبة الكلية ،و وحامض ±0.20 19.9 ±0.03 و 3.19 الاسكوربيك ،و % للنيتروجين ، و% للبروتين، على التوالى. كما كان هناك تأثير معنوى موجب للارتباط بين الصفات المختلفة.

نستنتج من هذه الدراسة بأنه للحصول على أعلى انتاج من البازلاء يوصى بمعاملة البذور بالمعاملة (رايزوبيوم + باسيللس + السماد النيتروجيني الفوسفاتي) .