

## Effect of *Rhizobium* Inoculation and Foliar Spray with Salicylic and Ascorbic Acids on Growth, Yield and Seed Quality of Pea plant (*Pisum sativum* L.) Grown on a Salt Affected Soil, New Valley-Egypt

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### Abstract

A field study was carried out to investigate the effects of seed inoculation with bio-fertilizer (*Rhizobium*), foliar spray with antioxidants i.e., salicylic acid (SA), ascorbic acid (AA) and SA+AA at 250 mg L<sup>-1</sup> of each and their interactions on growth, yield components and some seed bioconstituents of pea plant *Pisum sativum*, L cv. Master-B grown on a salt affected sandy loam soil (EC=9.65 ds m<sup>-1</sup>), New Valley-Egypt during seasons of 2015/2016 and 2016/2017 towards maximizing its productivity under salt stress conditions. Pea seeds were inoculated with *Rhizobium* as a bio-fertilizer source before planting. Pea seeds were inoculated at sowing with N fixing bacteria (*Rhizobium leguminosarum*). The application of antioxidants to plants was done as foliar spraying after twenty days after planting and repeated three times a ten days interval. The following data were recorded, vegetative growth parameters, root nodule characteristics, leaves photosynthetic pigments content, fresh as well as dry yield characteristics and seed chemical compositions. Inoculation significantly increased all the studied traits of pea plant grown under salinity conditions compared with un-inoculation. Foliar spraying of pea plants with antioxidants also, significantly increased all the studied aspects of pea plant, SA+AA was more efficient than spraying either solely. Inoculation seeds with *Rhizobium* and foliar spray of SA+AA significantly increased all the studied characteristics. SA+AA along with inoculation was more effective in increasing all the studied characteristics. Inoculation with *Rhizobium* along with foliar spraying with SA+AA could be recommended for growing pea under saline conditions.

**Keywords:** pea, *Rhizobium*, antioxidants, growth, yield and chemical compositions.

### Introduction

Pea (*Pisum sativum* L.) belongs to the family Fabaceae (Leguminosae). It is a vital legume vegetable, self-pollinated and hardy tendril climbing plant. It is a popular grain legume vegetable crop grown in Egypt and one of the major winter crops. It is the third most important legume in the world after soybean and common bean (*Phaseolus vulgaris* L.) (Timmerman-Vaughan *et al.*, 2005). It is one of the important sources in human food (Hussein *et al.*, 2006). It has high contents of amino acids, lysine and tryptophan, which relatively low in cereal grains. Pea seeds contain 21-25 % protein and up to 86 % of total digestible nutrients. It contains 5 to 20 % less trypsin inhibitor than soybean (Kent and Endres, 2003).

Soil salinization is a major factor limiting crop cultivation and production, (Paranychianakis and Chartzoulakis, 2005; Ahmed, 2009 and Shrivastava and Kumar, 2015). Plant productivity decreases due to osmotic inhibition of water uptake by roots or due to specific ion effects which may cause direct toxicity or imbalance of plant nutritional and salinity increases Na<sup>+</sup> uptake and decreases P and K<sup>+</sup> uptake, (Mayak *et al.*, 2004 and Yildirim *et al.*, 2006). Accumulation of excess Na<sup>+</sup> causes metabolic disturbance in plant, (Glenn *et al.*, 1999).

Salinity stress negatively affects growth, photosynthesis, mineral nutrition, stomatal

conductance, transpiration, water and ion transport and decreases sugars, amino acids and induces disorders as chlorosis, stunting leaves and impairing shoot growth, (Levy and Syvertsen, 2004). Steppuhn *et al.*, (2001) reported that under severe salinity; neither peas nor beans produced any grain. Salinity decreases water consumption of pea and decreases photosynthetic activity, (Jamil *et al.*, 2007).

Bio-fertilizers are preparation containing live or latent cells of efficient strains in N<sub>2</sub> fixation or P and K solubilization, (Vessey, 2003).

*Rhizobium leguminosarum* plays a principle role in N<sub>2</sub> fixation and can increase plant growth and yield, (Sobh *et al.*, 2000; El-Sheikh and Osman, 2002 and Tartoura, 2002). Inoculation of such strain would enhance nodulation and N<sub>2</sub> fixing, (Zahran, 1999 and Ali *et al.*, 2009).

Salicylic acid (SA) is a stress ameliorator (Hayat and Ahmad, 2007) and is an effective endogenous plant growth regulator, (Hayat *et al.*, 2010). It plays physiological roles in plant including growth, thermogenesis, flower induction, nutrients uptake, ethylene biosynthesis, stomata movement, photosynthesis and enzymes activity, (Hayat and Ahmad, 2007). It is involved in nodulation and senescence, (Khan *et al.*, 2003). It acts as regulator under salinity stress (Fahad and Bano, 2012; Iqbal *et al.*, 2014 and Fahad *et al.*, 2014).

Many investigations were carried out to protect plant against adverse effects of environmental stresses (Shokr and Abdelhamid, 2009) and ascorbic acid (AA) proved important as an antioxidant and enzyme cofactor and a cell signaling modulator, (Bolkhina *et al.*, 2003 and Wolucka *et al.*, 2005). It plays important role in improving plant tolerance to abiotic stress, (Shalata and Neumann, 2001; Al-Hakimi and Hamada, 2001 and Athara *et al.*, 2008). Its application to plants under salt stress increased chlorophyll content and stimulated growth and yield, (Buxton *et al.*, 2002; Dolatabadian and Jouneghani, 2009; Gad El-Hak *et al.*, 2012; Rady, 2013 and Mohsen *et al.*, 2014).

The present study was conducted on pea (*Pisum sativum* L.) cv. Master-B to investigate the effects of seed inoculation with biofertilizer (*Rhizobium*), foliar applications with two antioxidants, (salicylic acid and ascorbic acid) on growth, yield and its components as well as chemical constituents of pea grown on a salt affected soil, New Valley, Egypt.

## Materials and Methods

The field experiment involved bio-fertilization with or without foliar spray with antioxidants. The design was a randomized complete block, factorial (4 replicates) in a split-plot design. The main plots were assigned to bio-fertilization (2 treatments of none and *Rhizobium* inoculant). The sub-plots were assigned to foliar spray (4 treatments of no antioxidants" water spray," ascorbic acid, salicylic acid and a combination of both). The experiment was carried out at agricultural research farm of Faculty of Agriculture, Assiut University (New Valley Branch) during two successive winter seasons of 2015/2016 and 2016/2017. The soil was saline sandy loam. **Table 1** shows main properties of the soil. Soil analyses were according to the methods cited by (Black, 1965 and Page *et al.*, 1982).

**Table 1.** Physical and chemical properties of the experimental soil (from surface at 30 cm depth).

Property	Value	Property	Value
Particle size distribution		Soluble ions (mmol <sub>c</sub> L <sup>-1</sup> ) in Saturated soil paste extract	
C. Sand (%)	5.63	HCO <sub>3</sub> <sup>-</sup>	2.21
F. Sand (%)	76.19	CO <sub>3</sub> <sup>-2</sup>	0.00
Silt (%)	11.15	Cl <sup>-</sup>	5.79
Clay (%)	7.03	SO <sub>4</sub> <sup>-2</sup>	1.88
Soil texture (International texture triangle)	Sandy loam	Ca <sup>+2</sup>	1.98
Saturation percentage (%)	24.00	Mg <sup>+2</sup>	1.18
Field capacity (%)	12.00	Na <sup>+</sup>	6.23
Wilting point (%)	6.00	K <sup>+</sup>	0.49
Available water (%)	6.00	Available nutrients (mg kg <sup>-1</sup> )	
OM (g kg <sup>-1</sup> )	0.49	Available N (Kcl. Extract)	28.3
Ca CO <sub>3</sub> (g kg <sup>-1</sup> )	4.98	Available P (Na. biocarbonate extract)	4.76
pH*	8.05	Available K (Ammonium acetate extract)	115.90
EC* (ds m <sup>-1</sup> )	9.65		

\* EC and pH were measured in paste extract.

Pea seeds were inoculated with *Rhizobium* before planting. The sub-plot area was 14.70 m<sup>2</sup> and contained 6 ridges, with 3.50 m in length and 0.7 m in width. Concentration of salicylic acid as well as ascorbic acid was 250 mg/l of each.

### Time and method of treatments:

#### Bio-fertilizer (*Rhizobium*):

Pea seeds were inoculated at sowing. The seeds surface were sterilized with 1% sodium hypochlorite for 20 min and then rinsed with water several times. The surface of disinfected seeds was coated by soaking seeds in a liquid culture medium of *Rhizobium* for 15 minutes using 10% Arabic gum as adhesive, (Gabr *et al.*, 2007). Un-inoculated seeds were soaked in distilled water for 15 minutes also. The bio-fertilizer material was used at a rate of 20g/kg of seeds. Rhizobial population of the

inoculants used was 1.2 x 10<sup>8</sup> cell/cm<sup>3</sup> obtained from the Bio-fertilizer Unit, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Inoculum was prepared in flasks by using yeast extract mannitol broth medium Singh *et al.*, (2011) for *Rhizobium*. The inoculum was injected into sterile peat moss (100 ml inoculum per kg peat moss) and was incubated for 24 hrs. at 28±1°C before use. Seed dressing was carried out with the inoculum mixed with clay and 10% sugar solution. For the un-inoculated, seeds were coated with autoclaved broth and sugar solution.

#### Antioxidants:

Antioxidants (ascorbic and salicylic acid) were obtained from El-Gomhoria Company for Chemicals, Egypt. The application of these solutions to plants was done as foliar spray twenty days after planting and repeated three times a ten days interval throughout the growing seasons. The rate of spray

was 1200 L per hectare.

All plots were fertilized with cattle manure 70 m<sup>3</sup>/ha<sup>-1</sup> and 175 kg N/ha<sup>-1</sup> as ammonium sulphate (20.5% N) + 33 kg P/ha<sup>-1</sup> as calcium superphosphate (6.8% P) + 144 kg K/ha<sup>-1</sup> as potassium sulphate (40% K) and 240 kg sulphur/ha<sup>-1</sup> divided in four equal portions and the control of insects as well as pests were done according to the instructions of down by Ministry of Agriculture. Seeds were sown on 5<sup>th</sup> and 8<sup>th</sup> of November for 2015 and 2016 seasons, respectively. Seeds were sown in hills 10 cm apart, 2 seeds per hill at 3 cm depth on one side of the ridge. The fresh pods were harvested on 14<sup>th</sup> and 22<sup>nd</sup> of January for 2016 and 2017 seasons, respectively.

- 1. Vegetative growth characteristics:** Representative samples (ten plants per plot) were randomly taken at end of the growing season to determine the followings:
  - a. Shoot length (cm): Plants were uprooted carefully without damaging the root system. Shoot length was measured from the collar region to the tip of the plant.
  - b. Root length (cm): Roots of the uprooted plants were measured from the end region to the shoots of the plant to the tip of the roots.
  - c. Number of branches/plant.
  - d. Leaf area/plant (cm<sup>2</sup>) was measured using the disk method and calculated according to the formula of **Koller, (1972)**.
  - e. Fresh and dry weights of shoots (g).      f. Fresh and dry weights of roots (g).

#### **1. Chemical analysis:**

Bioconstituents were carried out in the samples of seeds during 2016 and 2017 seasons.

- a. **Photosynthetic pigments content:** The photosynthetic pigments chlorophyll A, B and carotenoids extracted from fresh leaf sample at 70 days after sowing by pure acetone and the pigments were filtered, the optical density of the filtrate was determined spectrophotometrically. The pigments content (as mg/g fresh weight) were calculated as adapted by **Wettstein, (1957)**.
- b. **Seed chemical compositions:** Representative seed samples at maturity stage from the second harvest were taken to determine the followings: **Total nitrogen** by the modified micro-Kjeldahl apparatus of Parnars and Wagner according to **Jones et al., (1991)** and **Crude protein** was calculated by multiplying N content by 6.25 according to **A.O.A.C., (2000)**. **Phosphorus** in sulphuric-perchloric acid digest, determined by a vanadate-molybdate method, **Chapman and Pratt, (1982)**. **Potassium** in acid digests, it was determined using a Perkin-elmer, Flame photometer according to **Page, (1982)**. **Total Carbohydrates** was determined according to **Mazumdar and Majumder, (2003)**. **Ascorbic acid** was determined by titration with 2.6 dichlorophenol indophenol blue dye according to the method described by **A.O.A.C., (2000)**. **Salicylic acid** was

determined photometrically using spectrophotometer (Backman OU.50) described by **Salinas et al., (1990)** and all determinations are calculated as %.

**3. Nodule characteristics:** Nodules of the samples were collected from roots manually, weighted and dried at 70 °C for 48 h to a constant weight to determine the number of root nodules/plant and the weight of nodules.

#### **4. Yield and its components:**

##### **4.1. Fresh yield characteristics:**

Fresh pods of 3 ridges in each treatment were harvested three times at 10 days interval started at the proper maturity stage (January 14<sup>th</sup> and 22<sup>nd</sup> in 2016 and 2017 seasons, respectively). Twenty pods were randomly taken from each plot from the second harvest and the following characteristics were calculated:

- a-Fresh pod length (cm).    b-Fresh pod diameter (cm).
- c- Fresh pod weight (g).
- d- Fresh seeds weight/pod (g).      e- Weight of 100 fresh seeds (g).      f- Number of fresh seeds/pod.
- g. Fresh pod yield (ton/ha) = 3 rows fresh pods yield (kg) × 10000 ÷ 0.5 plot area × 1000.

##### **4.2. Dry yield characteristics**

Dry pods of the other three ridges were harvested at the end of experiment. Twenty pods were randomly taken from each plot and the following parameters were determined:

- a. Average seeds weight/dry pod (g).    b. Weight of 1000 dry seeds (g).
- c. Dry seed yield (ton/ha) = 3 rows dry seeds yield (kg) × 10000 ÷ 0.5 plot area × 1000.

#### **5. Statistical analysis:**

Data were subjected to the statistical analysis of variance according to **Snedecor and Cochran, (1980)**. The treatment means were compared using Duncan's multiple range test at probability of 5 % according to **Duncan, (1958)**.

## **Results and Discussion**

### **1. Vegetative growth characteristics:**

Results in **Tables (2 and 3)** show the effects of inoculation of pea seeds with *Rhizobium* and foliar spray with antioxidants and their interactions on vegetative growth characteristics i.e., shoot length, root length (cm), number of branches/plant, leaf area/plant (cm<sup>2</sup>) as well as shoot, root fresh and dry weights (g) of pea plant grown under saline conditions.

#### **1.1. Effect of bio-fertilizer (*Rhizobium*):**

As for the inoculation with *Rhizobium*, the obtained results reveal that inoculating pea seeds with *Rhizobium* significantly increased all studied vegetative growth parameters as compared with uninoculation. This pattern of response occurred in both seasons of study. However, the increase in the studied vegetative growth aspects in the second

season was higher than that occurred in the first season and there is no significant difference between them. In this connection, (Gaballah and Gomaa, 2005) showed that *Rhizobium* inoculation of faba bean seeds reduced the inhibitory effect of salinity and plants were able to survive. (Gabr *et al.*, 2007) stated that inoculation of pea seeds with *Rhizobium* increased plant growth characteristics i.e., plant height, number of leaves and branches, plant fresh and dry weights. (Ullah *et al.*, 2016) mentioned that inoculation with *Rhizobium* increased root length, fresh and dry weights of mung bean plant under salt stress. (Benidire *et al.*, 2017) reported that inoculation of faba bean with the salt tolerant *Rhizobium leguminosarum* strains RhOF125 and RhOF34 increased plant biomass and alleviated toxic effects of salinity.

### 1.2. Effect of antioxidants:

Concerning the effect of foliar spray with the antioxidants i.e., salicylic acid (SA), ascorbic acid (AA) at 250 mg/liter of and their combination. Spraying plants with the antioxidants significantly increased all studied vegetative growth characteristics comparing with control (sprayed with water). This pattern of response occurred in both seasons of study. However, the increase in the studied vegetative growth characters in the second season was higher than that occurred in the first season and there was no significant differences between them. On the other hand, results show that spraying pea plants with the combination of salicylic and ascorbic acids gave higher values of growth traits than those obtained due to each alone. Thus, foliar spraying with the combination of salicylic and ascorbic acids was more effective in increasing vegetative growth characteristics than foliar spray of both individually. Same data also evidently confirmed the stimulatory effects of applied treatments upon dry matter production and accumulation in leaves and branches.

The effectiveness of used antioxidants in increasing growth aspects of pea plants grown under saline conditions could be supported by (Raskin, 1992) who found that SA plays an essential role in the regulation of plant growth and development. Salicylic acid is necessary for the induction of antioxidant defense and maintaining the redox state of the glutathione pool, (Borsani *et al.*, 2001). The increasing in growth parameters of salt affected plant in response to SA might be due to the protective role of SA on membranes which increases the tolerance of plants to salt stress (Aftab *et al.*, 2010). The SA mediated increase in growth of plants under salt stress could be to the antioxidant function and metabolic activity in plants (Gunes *et al.*, 2007). (Palma *et al.*, 2009) found that application with SA increased common bean dry weight and organic solutes content which indicates successful acclimatization of plants to saline conditions. (Gad

El-Hak *et al.*, 2012) stated that foliar spray with two antioxidants of SA, AA increased pea height, dry weight and number of branches. (Sakr *et al.*, 2013) investigated that applied AA and SA at 250 mg/l of each increased leaf area, stem and leaves dry weights of soybean under salinity stress. (Rady and Mohamed, 2015) investigated that foliar spray with SA improved shoot length, number and leaves area/plant and dry weight of common bean grown on a saline soil. (Sajid *et al.*, 2016) showed that increases in shoot/root growth, fresh/dry mass, leaf area and stem diameter of pea grown on pots by foliar spray with SA. (Shalata and Neumann, 2001) found that SA counteracted the salt induced in growth reduction. (Gabriela *et al.*, 2003) reported that AA caused positive effects on physiological processes including growth regulation, differentiation and metabolism of plants as well as fundamental its role in protecting metabolic processes against toxic effects of oxygen. (Kumari *et al.*, 2008) reported that vegetative growth of pea plant was enhanced by foliar AA. (Kamalendu and Srivastava, 2010) mentioned that pea growth increased with foliar spraying with SA. Some researchers reported that the highest plant growth was obtained from the SA foliar spray on pea (Farooq *et al.*, 2007) and on cowpea (Dhanavel and Girija, 2009). (Salama *et al.*, 2014) showed that foliar spray with AA mitigated the inhibitory effect of salt stress in kidney bean and induced a stimulatory effect on plant growth. (Barakat *et al.*, 2015) assumed that spraying AA to common bean grown under reclaimed soil conditions caused positive influences on dry weight and leaf/area plant.

### 1.3. Effect of the interaction between bio-fertilizer (*Rhizobium*) and antioxidants:

Respect to the effect of interactions between inoculating pea seeds with *Rhizobium* in combination with foliar spray with antioxidants on the vegetative growth parameters of pea grown in a saline soil, results indicate that inoculation of pea seeds along with foliar spraying with antioxidants of salicylic acid (SA), ascorbic acid (AA) individually or in combination at 250 mg/liter of each significantly increased growth parameters as compared with the sole application of *Rhizobium* and antioxidants. However, there was a significant interaction effect among the inoculation with *Rhizobium* along with foliar spray of antioxidants, where inoculation along with the combination of SA and AA gave the highest values of the vegetative growth aspects. This pattern of response occurred in both seasons of study. Such increments in growth aspects as a result of inoculation along with foliar spray of antioxidants may be attributed to the main roles of applied treatments in increasing the absorption of macro and micro nutrients from the soil and in turn affect plant vegetative growth. Such effect may be due to the role of these treatments in photosynthetic process which



consequently effect on the amounts of photosynthetic assimilates required for cells and tissues formation and in turn effect on morphological parameters of growing plant. Increasing of formed branches on a growing plant could be reversed upon many other characteristics as number of leaves, leaf area, leaves dry weight and finally pods yield.

The effectiveness of inoculation with *Rhizobium* along with the foliar spray of the antioxidants in

increasing growth characters of pea plant under saline conditions could be supported by (Zaki and Radwan, 2011) found that sprayed SA and AA at the rates of 150 mg L<sup>-1</sup> in combination with inoculation of wheat seeds with *Azotobacter chroococum* obtained highest wheat growth grown on a newly reclaimed soil under saline stress.

**Table 2.** Vegetative growth characteristics of pea plant grown on a saline soil as affected by *Rhizobium*, antioxidants and their interactions during 2015/2016 and 2016/2017 growing seasons.

Treatments	2015/2016					2016/2017				
	Control	SA	AA	SA + AA	Mean	Control	SA	AA	SA + AA	mean
Shoot length (cm)										
- <i>Rhizobium</i>	25.40 f	28.90 e	27.80 e	28.90 e	27.75 b	25.70 f	29.00 e	28.70 e	33.80 d	29.30 b
+ <i>Rhizobium</i>	31.20 d	35.60 b	33.30 c	38.10 a	34.55 a	34.50 cd	37.30 b	36.10 bc	39.40 a	36.82 a
mean	28.30 c	32.25 a	30.55 b	33.50 a		30.10 c	33.15 b	32.40 b	36.60 a	
Root length (cm)										
- <i>Rhizobium</i>	12.48 f	16.29 e	15.18 e	18.27 d	15.55 b	13.50 e	17.11 d	16.64 d	20.28 c	16.88 b
+ <i>Rhizobium</i>	19.78 cd	23.77 b	21.14 c	26.58 a	22.82 a	21.46 c	25.60 b	24.63 b	28.64 a	25.08 a
mean	16.13 d	20.03 b	18.16 c	22.42 a		17.48 c	21.35 b	20.63 b	24.46 a	
Number of branches/plant										
- <i>Rhizobium</i>	1.20 e	1.40 e	1.50 de	1.90 cd	1.50 b	1.20 d	1.50 d	1.60 d	2.10 c	1.60 b
+ <i>Rhizobium</i>	2.10 bc	2.40 b	2.50 ab	2.90 a	2.47 a	2.30 bc	2.40 bc	2.60 b	3.10 a	2.60 a
mean	1.65 c	1.90 bc	2.00 b	2.40 a		1.75 c	1.95 bc	2.10 b	2.60 a	
Leaf area/plant (cm <sup>2</sup> )										
- <i>Rhizobium</i>	22.04 f	26.36 e	27.87 de	29.13 cd	26.35 b	25.06 f	27.16 e	27.92 de	29.50 d	27.41 b
+ <i>Rhizobium</i>	30.62 bc	32.06 b	32.30 ab	33.85 a	32.21 a	31.34 c	33.65 b	34.12 ab	35.55 a	33.66 a
mean	26.33 c	29.21 b	30.08 b	31.49 a		28.20 c	30.41 b	31.02 b	32.53 a	

Values followed by same letters within a column not significantly differed at 5% according to Duncan's multiple range test. SA: Salicylic acid at 250 mg/L. and AA: Ascorbic acid at 250 mg/L.

**Table 3.** Fresh and dry weights (g) of pea plant shoot and root as affected by *Rhizobium*, antioxidants and their interactions during 2015/2016 and 2016/2017 growing seasons.

Treatments	2015/2016					2016/2017				
	Control	SA	AA	SA + AA	Mean	Control	SA	AA	SA + AA	mean
Shoot fresh weight										
- <i>Rhizobium</i>	8.09 e	9.85 d	10.65 cd	11.81 bc	10.10 b	8.49 e	10.55 d	12.02 cd	12.71 bc	10.94 b
+ <i>Rhizobium</i>	13.13 b	15.02 a	15.38 a	16.58 a	15.03 a	13.99 b	15.92 a	16.18 a	16.88 a	15.74 a
mean	10.61 c	12.43 b	13.01 ab	14.19 a		11.24 c	13.23 b	14.10 ab	14.79 a	
Root fresh weight										
- <i>Rhizobium</i>	3.05 f	3.50 e	3.51 e	3.96 d	3.50 b	3.13 f	3.68 e	3.84 e	4.19 d	3.71 b
+ <i>Rhizobium</i>	4.68 c	5.25 b	5.29 b	5.93 a	5.29 a	5.10 c	5.54 b	5.58 b	5.96 a	5.54 a
mean	3.86 c	4.38 b	4.40 b	4.95 a		4.11 c	4.61 b	4.71 b	5.07 a	
Shoot dry weight										
- <i>Rhizobium</i>	3.14 f	3.30 f	3.52 e	3.79 d	3.44 b	3.16 e	3.30 e	4.03 d	4.19 cd	3.67 b
+ <i>Rhizobium</i>	4.05 c	4.35 b	4.39 ab	4.57 a	4.34 a	4.35 bc	4.45 b	4.69 a	4.87 a	4.59 a
mean	3.59 c	3.82 b	3.96 b	4.18 a		3.75 c	3.87 c	4.36 b	4.53 a	
Root dry weight										
- <i>Rhizobium</i>	0.94 d	1.06 cd	1.07 cd	1.18 bc	1.06 b	1.04 d	1.09 d	1.13 d	1.38 c	1.16 b
+ <i>Rhizobium</i>	1.30 b	1.52 a	1.53 a	1.69 a	1.51 a	1.57 b	1.61 b	1.64 ab	1.80 a	1.66 a
mean	1.12 c	1.29 b	1.30 b	1.43 a		1.30 b	1.35 b	1.38 b	1.59 a	

Values followed by same letters within a column not significantly differed at 5% according to Duncan's multiple range test. SA: Salicylic acid at 250 mg/L. and AA: Ascorbic acid at 250 mg/L.

## 2. Photosynthetic pigments content:

Data in **Table (4)** indicate the effect of inoculation pea seeds with *Rhizobium* and applied antioxidants i.e., SA, AA and SA+AA at 250 mg/liter of each individually or in combination treatments on the photosynthetic pigments content of chlorophyll A, B and carotenoids in pea plant leaves grown under saline conditions at 70 days after sowing during both seasons.

### 2.1. Effect of bio-fertilizer (*Rhizobium*):

As for the effect of *Rhizobium*, data in **Table (4)** show that inoculating seeds with *Rhizobium* increased the content of photosynthetic pigments of pea plants comparing with un-inoculated pea. In other words, the control treatment (un-inoculated seeds) under saline conditions gave the highest reduction in chlorophyll A, B and carotenoids of pea plants. These results could be explained by (**Gabr et al., 2007**) investigated that inoculation of pea with *Rhizobium* increased content of chlorophyll compared to un-inoculated. (**Bambara and Ndakidemi, 2009**) indicated that inoculation of *Phaseolus vulgaris* seeds with *Rhizobium* affected on chlorophyll content and the intercellular CO<sub>2</sub> concentration compared to *Rhizobium* un-inoculated. (**Chame et al., 2016**) showed that inoculation of bean seeds with biofertilizer had a significant effect on chlorophyll index compared to non-inoculated.

### 2.2. Effect of antioxidants:

Concerning the effect of applied antioxidants of SA, AA individually or in combination increased the content of each chlorophyll A, B and carotenoids in pea leaves at 70 days after sowing during 2016 and 2017 seasons compared to the control (sprayed with water). It could be noticed that maximum increases of these pigments content were existed with the combination between SA and AA treatment at 250 mg/liter of each followed by SA and AA solely treatments, respectively. It was obvious that control plants were negatively affected, as they were physiologically stressed. That might be due to they were not developed any mechanism by which they could be protected against prevailing saline stress and its probable inducible oxidative one (**Marschner, 1995**).

In this respect, the stimulation of photosynthetic pigments formation could be attributed to the vigorous growth obtained in **Tables (2 and 3)**. Increasing of chlorophylls and carotenoids content in pea leaves as affected by applied antioxidants may be due to enhancing photosynthetic efficiency and increasing dry matter production. Our results could be supported by (**McKersie et al., 1996**) documented that the photosynthetic apparatus is a target to reactive oxygen species. Under stress conditions, excess ROS attack lipids, protein and nucleic acids, resulted in lipid peroxidation, protein inactivation as

well as DNA alteration. They also reported that plants with high level of antioxidants, whether induced a greater resistance to oxidative stress. (**El-Shraiy and Hegazi, 2009**) found that foliar spray with AA on pea correlated with increasing leaves chlorophyll content. (**Aftab et al., 2010**) reported that increasing in photosynthesis of salt affected *Artemisia annua* L. as a response to SA treatment may be due to the protective role of SA on membranes that may increase plants tolerance to salt stress and metabolic activity. Ascorbic acid is a major antioxidant in plant known to increase plant growth and cell cycle, through photosynthetic apparatus and plant protecting of any ROS and increasing Rubisco subunit, photosynthetic pigments thereby increasing photosynthetic rate and productivity (**Chen and Gallie, 2006**). (**Osman et al., 2014**) demonstrated that foliar application with AA increased values of chlorophyll A, B content of sunflower plant. (**Salama et al., 2014**) investigated that AA foliar spray not only mitigated the inhibitory effects of salt stress on kidney bean but also accompanied by increasing in photosynthetic system.

### 2.3. Effect of the interaction between bio-fertilizer (*Rhizobium*) and antioxidants:

As for the effect of interactions, data in **Table (4)** clearly indicate that the interaction effects between inoculated pea seeds with *Rhizobium* and applied antioxidants i.e., salicylic and ascorbic acids as well as their combination at 250 mg/liter of each treatments increased the concentration of chlorophyll A, B and carotenoids in leaves of pea plants at 70 days after sowing in 2015/2016 and 2016/2017 seasons under saline conditions. The same data cleared that the most effective treatment which led to maintained the highest concentrations of determined photosynthetic pigments was the inoculation of pea along with SA+AA followed by inoculated pea in combination with SA and *Rhizobium* combined with AA treatments, respectively. The same data also, evidently confirmed the stimulatory effects of different applied treatments upon dry matter production and accumulation in leaves and branches as shown in **Tables (2 and 3)**. In general, data in **Table (4)** not only being a direct results for that vigorous growth obtained in **Tables (2 and 3)** but also could be considered an indicator for expectable high yield of pods and seeds. It was obvious from the same data the stimulatory effects of applied treatments in improving the photosynthetic pigments content and alleviated adverse effects of saline stress and its probable accompanied oxidative stress on treated pea plants may be due to their antioxidant scavenging effects in protecting plant form degradation by toxic Reactive Oxygen Radicals which internally generated during stress, (**Conklin, 2001**).

**Table 4.** Photosynthetic pigments content (mg/g F.W.) of pea plant leaf as affected by *Rhizobium*, antioxidants and their interactions during 2015/2016 and 2016/2017 growing seasons.

Treatments	2015/2016					2016/2017				
	Control	SA	AA	SA + AA	mean	Control	SA	AA	SA + AA	mean
Chlorophyll (A)										
- <i>Rhizobium</i>	0.53 f	0.81 de	0.74 e	0.87 d	0.74 b	0.47 h	0.52 g	0.58 f	0.67 e	0.56 b
+ <i>Rhizobium</i>	0.86 d	1.03 c	1.18 b	1.41 a	1.12 a	1.13 d	1.22 c	1.30 b	1.54 a	1.30 a
mean	0.69 c	0.92 b	0.96 b	1.14 a		0.80 d	0.87 c	0.94 b	1.10 a	
Chlorophyll (B)										
- <i>Rhizobium</i>	0.12 g	0.19 d	0.21 c	0.13 f	0.16 b	0.11 h	0.19 e	0.14 f	0.12 g	0.14 b
+ <i>Rhizobium</i>	0.18 e	0.28 b	0.21 c	0.34 a	0.25 a	0.33 c	0.36 b	0.27 d	0.40 a	0.34 a
mean	0.15 c	0.23 a	0.21 b	0.23 a		0.22 c	0.27 a	0.20 d	0.26 b	
Carotenoids										
- <i>Rhizobium</i>	0.37 h	0.42 g	0.46 e	0.51 d	0.44 b	0.41 g	0.57 d	0.55 e	0.62 c	0.54 b
+ <i>Rhizobium</i>	0.45 f	0.73 a	0.58 b	0.57 c	0.58 a	0.35 h	0.64 b	0.53 f	0.75 a	0.57 a
mean	0.41 d	0.57 a	0.52 c	0.54 b		0.38 d	0.60 b	0.54 c	0.68 a	

Values followed by same letters within a column not significantly differed at 5% according to Duncan's multiple range test. SA: Salicylic acid at 250 mg/L. and AA: Ascorbic acid at 250 mg/L.

### 3. Nodule characteristics:

As shown in **Table (5)** data indicate the effect of different applied treatments i.e., inoculation pea seeds with *Rhizobium* and foliar application with antioxidants i.e., SA, AA and SA+AA at 250 mg/liter of each individually or in combination on number of root nodules and nodule fresh as well as dry weights (g) of pea plants grown under saline conditions during both seasons.

#### 3.1. Effect of bio-fertilizer (*Rhizobium*):

Concerning the effect of *Rhizobium*, the obtained results in **Table (5)** clearly reveal that inoculating pea seeds with *Rhizobium* treatment significantly increased number of root nodules, nodule fresh and nodule dry weights (g) aspects as compared with the control (un-inoculation). This pattern of response occurred in the both seasons of study. In this respect, the obtained increases of these traits are of great interest because these data go well with the previously mentioned results i.e., estimated growth characteristics, **Tables (2 and 3)** as well as photosynthetic pigments content, **Table (4)** and will answer many questions especially about increasing yielded pods of treated pea plants. These significantly favorable effects of inoculation pea seeds with *Rhizobium* treatment may be explained on the basis that the beneficial effects of increasing number of root bacterial nodules and their weights on the nutrient availability, vital enzymes and hormonal stimulating effects may provide principal evident for this view.

These results are in conformity with those obtained by (**Zahran, 1999**) reported that salinity important stress affecting rhizobial legume symbiosis. (**Slatery et al., 2001**) indicated that salinity reducing population of *Rhizobium* in the soil and high number of naturally occurring *Rhizobium* population was poor in its ability to fix N and can compete strongly with introduced *Rhizobium* inoculum. Salinity inhibited nodulation and N fixation in *Pisum sativum* L. inoculated with

*Rhizobium leguminosarum*, this symbiosis is extremely sensitive to salt (**El-Hamdaoui et al., 2003**). (**Gaballah and Gomaa, 2005**) cleared that salinity reduced nodules formation (No. of nodules/plant and nodule dry weight) in faba bean. This effect was limited by *Rhizobium* inoculation. (**Mishra et al., 2010**) stated that highest number and dry weight of nodules per plant found by seed inoculation with *Rhizobium* in pea. (**Aamir et al., 2013**) illustrated that inoculation mung bean seeds with rhizobia increased No. of root nodules, nodule fresh and dry weights under salinity conditions. (**Benidire et al., 2017**) suggested that inoculation with salt tolerant *Rhizobium leguminosarum* increased root nodules No. in *V. faba* L. plant and seemed to protect faba bean against the toxic effects of salt stress.

#### 3.2. Effect of antioxidants:

Regarding, the effect of foliar application with antioxidants, i.e., salicylic acid, ascorbic acid at 250 mg/liter of each and their combination, the obtained results show that spraying pea plants with antioxidants significantly increased bacterial nodule characteristics comparing with control. On the other hand, results show that spraying pea plant with the combination of salicylic and ascorbic acids gave the highest values of bacterial nodule traits comparing with those obtained due to the foliar spray with salicylic or ascorbic solely. Thus, foliar spray with the combination of salicylic and ascorbic acids was more effective in increasing these aspects. Same data also evidently confirmed the stimulatory effects of applied treatments upon increment of nodulation in pea plant grown under saline conditions and seemed to protect pea plants against the toxic effects of salinity. The same data also, proved that salinity reduced pea root nodulation; this effect significantly limited by foliar application with antioxidants.

These results could be supported by (**Rasmussen et al., 1991**) reported that SA plays major roles at the

early stages of *Rhizobium* legume symbiosis. (El-Shraiy and Hegazi, 2009) indicated that application with SA on pea achieved the maximum nodules No./plant. (Ismaeil and Abd El-Ail, 2011) found that foliar application with salicylic and ascorbic acids increased root nodules number of faba bean plant.

### 3.3. Effect of the interaction between bio-fertilizer (*Rhizobium*) and antioxidants:

As for the effect of interactions, data in Table (5) clearly show that the interaction effects between inoculated pea seeds with *Rhizobium* and applied antioxidants i.e., SA and AA acids as well as their combination at 250 mg/liter of each treatments increased number of root nodules, nodule fresh and dry weights of pea plant during 2015/2016 and

2016/2017 seasons under saline conditions. The same data also, indicate that inoculation with *Rhizobium* along with foliar spray with the combination of SA and AA was the most effective treatment which gave the highest values of nodulation aspects. These significantly favorable effects of the combination between *Rhizobium* and antioxidants may be explained on the basis of the beneficial effects of bacteria on the nutrient availability and hormonal stimulating effects on pea plant from side and the roles of applied antioxidants to protect pea plants against the toxic effects of salinity from other side. Increment of nodulation aspects is of great interest since that could be reflected upon estimated growth characteristics and bio constituents as well as yield of pea plants grown under saline conditions.

**Table 5.** Number and fresh as well as dry weights (g) of pea root nodule as affected by *Rhizobium*, antioxidants and their interactions during 2015/2016 and 2016/2017 growing seasons.

Treatments	2015/2016					2016/2017				
	Control	SA	AA	SA + AA	mean	Control	SA	AA	SA + AA	mean
Number of root nodules/plant										
- <i>Rhizobium</i>	2.20 d	3.40 c	3.30 c	3.70 c	3.15 b	2.50 e	3.60 d	3.60 d	4.20 d	3.47 b
+ <i>Rhizobium</i>	9.90 b	11.80 a	11.60 a	12.20 a	11.37 a	10.90 c	14.20 ab	13.50 b	14.70 a	13.32 a
mean	6.05 b	7.60 a	7.45 a	7.95 a		6.70 c	8.90 ab	8.55 b	9.45 a	
Nodule fresh weight										
- <i>Rhizobium</i>	0.182 f	0.204 e	0.202 e	0.250 d	0.209 b	0.198 g	0.257 e	0.236 f	0.325 d	0.254 b
+ <i>Rhizobium</i>	0.356 c	0.409 a	0.382 b	0.409 a	0.389 a	0.388 c	0.492 a	0.445 b	0.504 a	0.457 a
mean	0.269 d	0.306 b	0.292 c	0.329 a		0.293 d	0.374 b	0.340 c	0.414 a	
Nodule dry weight										
- <i>Rhizobium</i>	0.016 e	0.019 d	0.017 e	0.022 c	0.018 b	0.016 f	0.020 e	0.019 e	0.027 d	0.021 b
+ <i>Rhizobium</i>	0.028 b	0.034 a	0.034 a	0.035 a	0.033 a	0.033 c	0.040 a	0.038 b	0.040 a	0.038 a
mean	0.022 c	0.026 b	0.025 b	0.028 a		0.025 c	0.030 b	0.029 b	0.034 a	

Values followed by same letters within a column not significantly differed at 5% according to Duncan's multiple range test. SA: Salicylic acid at 250 mg/L. and AA: Ascorbic acid at 250 mg/L.

## 4. Yield and its components:

As for the effect of different applied treatments, data in Tables (6 and 7) clearly indicate that each of inoculation of pea seeds with *Rhizobium*, foliar application of antioxidants and their interaction treatments had significant effects on studied fresh as well as dry yield characteristics i.e., pod length, pod diameter (cm), fresh pod weight, fresh seeds weight, weight of 100 fresh seeds (g), number of fresh seeds/pod, seeds weight/dry pod, weight of 1000 dry seeds (g), fresh pod yield and dry seed yield ton/ha of pea plants grown under saline conditions during both seasons.

### 4.1. Effect of bio-fertilizer (*Rhizobium*):

Regarding, the effect of inoculation, the obtained results show that inoculating pea seeds with *Rhizobium* significantly increased fresh as well as dry yield characteristics of pea plants as compared with un-inoculation in both seasons.

These results are in agreement with those of (Shafeek *et al.*, 2004) on bean and (Abou El-

Salehein *et al.*, 2005) on pea observed that inoculation with *Rhizobium* improved pod characteristics. (Gaballah and Gomaa, 2005) reported that used *Rhizobium* inoculation on faba bean seeds obtained yield increasing. (Ahmed *et al.*, 2007) found that used inoculation with *Rhizobium* on pea obtained increases in number of pods/plant, pod length/plant, number of seeds/pod, 1000 fresh and mature seed weights, fresh pod and mature seed yield. (El Sayed *et al.*, 2012) reported that pea seeds inoculated with *Rhizobium* increased yield traits i.e., pod length, pod weight, number of fresh seeds/pod, weight of 100 fresh seed and seed index.

These present results show that bacterial inoculants, favored the following beneficial effects, N<sub>2</sub> fixation, acquisition of P, increasing availability of nutrients and producing adequate amounts of Auxins and Cytokinins which affect growth, morphological and physiological aspects of root, (Martin *et al.*, 1989 and Jagnow *et al.*, 1991).



#### 4.2. Effect of antioxidants:

As for the effect of antioxidants, the recorded data reveal that spray pea plants with SA, AA and their combination significantly increased fresh and dry yield components of pea plant as compared with non-sprayed. There was no significant difference between SA and AA in increasing yield aspects except fresh pod weight. The highest yield characteristic values were obtained by foliar spray with the combination of SA and AA. There was a significant difference between the effect of foliar spray with the combination of SA and AA in increasing yield traits and foliar spray with each of SA and AA individually, except weight of 100 fresh seeds. Consequently, foliar spray with the combination of salicylic and ascorbic acids was more effective than foliar spray of both individually. Such increments in yield aspects as a result of using tested antioxidants may be due to the main role of antioxidants on enzymatic reactions in plant metabolism and its role in catching and binding as well as scavenging of ROS which affect on plant metabolism and consequently increased plant yield. The increasing in plant yield traits may be attributed to increasing of photosynthetic pigments, absorption of nutrients and their effects on different physiological and chemical reactions in plant which positively effect on plant yield (Marschner, 1995).

The effectiveness of studied antioxidants in increasing yield characteristics of pea plant grown under saline conditions could be supported by (Ghulam, 2007) reported that foliar spray pea with SA increased yield aspects. (El-Hendaway *et al.*, 2011) concluded that faba bean yield increased with foliar spray of SA. (Radi *et al.*, 2001 and Ebrahim, 2005) reported that AA increased dry matter yield of salt stressed bean. (Helal *et al.*, 2005) on pea plant concluded that spray with AA increased yield. This may be due to increases in endogenous promoter phytohormones, in addition stimulating plant growth and CO<sub>2</sub> as well as microbial activity (Abd El-Halim, 1995). These results confirm the positive effects of spray with AA on influencing several physiological process as stimulating cell division and several enzymes activities (Abd-El-Hamed *et al.*, 2004; Irfan *et al.*, 2006 and Zewail, 2007). AA inhibits stress induced leakage of essential electrolytes following peroxidative damage for plasma membrane (McKersie *et al.*, 1999). The effect of AA in mitigating the adverse effects of salt stress may be due to the activation of enzymatic reactions. (El-Shraiy and Hegazi, 2009) reported that obtained increases in 1000 seed weight and pod yield of pea by foliar spray with SA. (Chame *et al.*, 2016) stated that sprayed beans with SA increases 100 seeds weight and grain yield under salinity. The effect of foliar spray with antioxidants on increasing

fresh pod yield of pea could be a result to the effect on improving plant growth (Gad El-Hak *et al.*, 2012). These results are in agreement with (Gahalain *et al.*, 1999; Alqurainy, 2007 and Yadav *et al.*, 2008) on pea and (Sultana *et al.*, 2009) on faba bean. (Khafaga *et al.*, 2014) showed that foliar application of AA increased faba bean productivity.

#### 4.3. Effect of the interaction between bio-fertilizer (*Rhizobium*) and antioxidants:

With regard to the effect of inoculation along with foliar spray of antioxidants on fresh and dry yield characteristics of pea plant grown in a saline soil, results indicate that inoculation of pea seeds with *Rhizobium* along with foliar spray with antioxidants significantly increased yield aspects as compared with the sole application of both. There was a significant difference between the combination of SA+AA foliar spray along with inoculation and the effect of foliar spray of SA or AA along with inoculation. The highest yield characteristics were obtained due to spray SA+AA along with inoculation. The effectiveness of inoculation along with foliar spray of antioxidants in increasing yield aspects could be arranged as *Rhizobium*+combination of SA+AA was the most superior treatment followed by the combination of *Rhizobium* and SA then the combination of *Rhizobium* and AA treatments, respectively.

It was obvious from the data that control plant was strongly stressed. Incongruity, the different applied treatments increased plant yield aspects as a direct result for the simulative effect of these treatments, that actively enhanced the internal metabolically protective status by their direct scavenging functions against the toxic free radicals (induced by saline stress) or due to their promotional effect on synthesis of natural protective antioxidants, **Table (9)** i.e., phenols, sugars, carotenoids and so on as well as they induce an potent biosynthesis case due to the higher photosynthetic pigments content as shown in **Table (4)**, protection of chlorophyll and chloroplasts against stress degradable effects from a side as well as increasing the absorption root surface for water and nutrients which consequently affect positively on plant growth, **Tables (2 and 3)** from other side, there by higher carbohydrates accumulation and content as well as higher minerals N, P and K content, **Table (8)** which finally resulting in increasing plant yield, **Tables (6 and 7)**. The strong positive correlations of such constituent vs. seeds yield confirm and coincide such functions of antioxidants under saline stress. (Chame *et al.*, 2016) reported that inoculating bean seeds with biofertilizer and using SA alleviated the effects of salinity on yield.

**Table 6.** Yield characteristics of pea plant as affected by *Rhizobium*, antioxidants and their interactions during 2015/2016 and 2016/2017 growing seasons.

Treatments	2015/2016					2016/2017				
	Control	SA	AA	SA + AA	mean	Control	SA	AA	SA + AA	mean
Fresh pod length (cm)										
- <i>Rhizobium</i>	7.35 e	7.59 d	7.53 de	7.87 c	7.58 b	7.44 f	7.68 ef	7.75 e	8.06 d	7.73 b
+ <i>Rhizobium</i>	8.07 c	8.55 b	8.33 b	8.88 a	8.45 a	8.26 cd	8.63 ab	8.48 bc	8.91 a	8.57 a
mean	7.71 c	8.07 b	7.93 b	8.37 a		7.85 c	8.15 b	8.11 b	8.48 a	
Fresh pod diameter (cm)										
- <i>Rhizobium</i>	1.02 d	1.05 cd	1.05 cd	1.09 bc	1.05 b	1.02 e	1.06 de	1.07 de	1.09 d	1.06 b
+ <i>Rhizobium</i>	1.10 bc	1.14 b	1.13 b	1.21 a	1.14 a	1.11 cd	1.15 bc	1.17 b	1.24 a	1.16 a
mean	1.06 b	1.09 b	1.09 b	1.15 a		1.06 c	1.10 b	1.12 b	1.16 a	
Fresh pod weight (g)										
- <i>Rhizobium</i>	5.90 e	6.52 d	6.17 e	6.53 d	6.28 b	6.21 e	6.58 d	6.55 d	6.76 cd	6.53 b
+ <i>Rhizobium</i>	6.68 cd	7.34 b	6.87 c	7.65 a	7.13 a	6.97 bc	7.56 a	7.05 b	7.77 a	7.34 a
mean	6.29 c	6.93 a	6.52 b	7.09 a		6.59 c	7.07 a	6.80 b	7.26 a	
Fresh seeds weight/pod (g)										
- <i>Rhizobium</i>	2.29 f	2.64 de	2.55 e	2.81 d	2.57 b	2.30 f	2.65 e	2.62 e	2.87 d	2.61 b
+ <i>Rhizobium</i>	3.05 c	3.49 b	3.37 b	3.79 a	3.42 a	3.06 c	3.56 b	3.45 b	3.89 a	3.49 a
mean	2.67 c	3.06 b	2.96 b	3.30 a		2.68 c	3.11 b	3.03 b	3.38 a	
Weight of 100 fresh seeds (g)										
- <i>Rhizobium</i>	38.26 d	40.85 cd	40.67 cd	41.52 cd	40.33 b	38.41 e	41.10 de	41.08 de	41.82 d	40.60 b
+ <i>Rhizobium</i>	42.48 bc	46.12 a	45.32 ab	48.65 a	45.64 a	42.66 cd	46.47 ab	45.64 bc	49.29 a	46.01 a
mean	40.37 b	43.48 a	43.00 a	45.09 a		40.53 b	43.79 a	43.36 a	45.55 a	

Values followed by same letters within a column not significantly differed at 5% according to Duncan's multiple range test. SA: Salicylic acid at 250 mg/L. and AA: Ascorbic acid at 250 mg/L.

**Table 7.** Yield characteristics of pea plant as affected by *Rhizobium*, antioxidants and their interactions during 2015/2016 and 2016/2017 growing seasons.

Treatments	2015/2016					2016/2017				
	Control	SA	AA	SA + AA	mean	Control	SA	AA	SA + AA	mean
Number of fresh seeds/pod										
- <i>Rhizobium</i>	6.00 f	6.50 de	6.30 ef	6.80 cd	6.40 b	6.00 d	6.50 c	6.40 c	6.90 b	6.45 b
+ <i>Rhizobium</i>	7.20 bc	7.60 ab	7.50 ab	7.80 a	7.52 a	7.20 b	7.70 a	7.60 a	7.90 a	7.60 a
mean	6.60 c	7.05 ab	6.90 b	7.30 a		6.60 c	7.10 b	7.00 b	7.40 a	
seeds weight/dry pod (g)										
- <i>Rhizobium</i>	0.36 f	0.62 e	0.58 e	0.75 d	0.58 b	0.39 f	0.77 e	0.71 e	0.89 d	0.69 b
+ <i>Rhizobium</i>	1.09 c	1.38 b	1.33 b	1.67 a	1.37 a	1.13 c	1.46 b	1.38 b	1.71 a	1.42 a
mean	0.72 c	1.00 b	0.96 b	1.21 a		0.76 c	1.12 b	1.04 b	1.30 a	
Weight of 1000 dry seeds (g)										
- <i>Rhizobium</i>	60.16 f	96.14 e	93.19 e	111.40 d	90.22 b	65.83 f	119.85 de	111.30 e	130.59 d	106.89 b
+ <i>Rhizobium</i>	152.41 c	182.08 b	179.25 b	215.00 a	182.18 a	157.96 c	191.73 b	182.64 b	217.00 a	187.33 a
mean	106.28 c	139.11 b	136.22 b	163.20 a		111.89 c	155.79 b	146.97 b	173.79 a	
Fresh pod yield (ton/ha)										
- <i>Rhizobium</i>	3.20 f	4.67 de	4.21 e	5.30 d	4.35 b	3.92 f	4.83 e	4.68 e	5.53 d	4.74 b
+ <i>Rhizobium</i>	6.25 c	7.87 ab	7.21 b	8.38 a	7.43 a	6.39 c	7.93 b	7.73 b	8.56 a	7.65 a
mean	4.73 d	6.27 b	5.71 c	6.84 a		5.15 c	6.38 b	6.20 b	7.05 a	
Dry seed yield (ton/ha)										
- <i>Rhizobium</i>	1.02 f	1.25 e	1.17 e	1.43 d	1.22 b	1.15 f	1.44 d	1.32 e	1.45 d	1.34 b
+ <i>Rhizobium</i>	1.52 c	1.65 b	1.60 b	1.75 a	1.63 a	1.55 c	1.73 ab	1.66 b	1.78 a	1.68 a
mean	1.27 d	1.45 b	1.39 c	1.59 a		1.35 c	1.58 a	1.49 b	1.61 a	

Values followed by same letters within a column not significantly differed at 5% according to Duncan's multiple range test. SA: Salicylic acid at 250 mg/L. and AA: Ascorbic acid at 250 mg/L.

## 5. Seed chemical compositions:

Data in **Tables (8 and 9)** clearly show the effect of the inoculation of pea seeds with *Rhizobium* and foliar spray of pea plant with antioxidants as well as their combinations on some bioconstituents of N, P, K, crude protein, total carbohydrates, ascorbic and salicylic acids % during both seasons.

### 5.1. Effect of bio-fertilizer (*Rhizobium*):

Concerning the effect of inoculation of pea seeds with *Rhizobium*, it could be noticed that inoculating significantly increased chemical compositions of pea seeds as compared with un-inoculation in both seasons of study.

Similar results were reported by (Marschner, 1995) indicated that increasing seed bioconstituents is a result of N fixation by *Rhizobium* from the atmospheric N in root media, since more than 90% of fixed N translocate from bacteria to the different organs. (Ahmed *et al.*, 2007) stated that inoculation with *Rhizobium* performed best recording in protein pea seed content. (El Sayed *et al.*, 2012) showed that pea seeds inoculated with *Rhizobium* increased N, P, K, carbohydrates and protein of fresh seeds. (Aamir *et al.*, 2013) observed that inoculation of mung bean with *Rhizobium* increased N, P, K and protein content in seeds under salinity conditions.

### 5.2. Effect of antioxidants:

Regarding to the effect of foliar spray with antioxidants i.e., SA and AA as well as their combination, the obtained results show that spray pea plant with antioxidants significantly increased N, P, K, crude protein, total carbohydrates, ascorbic and salicylic acids % of pea seeds as compared with untreated plant.

In other words, different applied treatments individually or in combination increased determined biochemical compositions of pea seeds grown under saline conditions. In this respect, combination between SA and AA gave the highest values followed by SA and AA solely treatment, respectively when compared with control.

These results are in harmony with (Raskin, 1992) documented that phenolic compounds exert their influence on physiological and biochemical processes including photosynthesis, ions uptake, enzyme activities and so on. SA is a natural compound may be function as a plant growth regulator (Arberg, 1981). Not only SA could be recommended to improve plant growth and productivity but also to induce plant resistance against the biotic and abiotic stresses as well, (Raskin, 1995). (Haroun *et al.*, 1998) reported that SA increased total carbohydrates content in lupine seed. (Sarang *et al.*, 2003) showed that N and protein contents increased in *Phaseolus vulgaris* with foliar spray of SA. SA increases uptake of potassium under salinity stress, potassium being essential to protein synthesis (Kim *et al.*, 2005). Salicylic acid enhanced antioxidant system and reduces the destructive effects of ROS (Ghorbani *et al.*, 2004).

(Trachevsky *et al.*, 2011) mentioned that foliar spray with SA on pea produced high protein content and P %. (Hadi *et al.*, 2014) showed that SA was the most effective treatment on protein content of white bean seed and reduced adverse effects of salinity. (Gad El-Hak *et al.*, 2012) reported that foliar spray with AA affected N, protein and P in pea seeds. (Talaat, 2003) stated that nitrate accumulation by foliar spray with AA may be due to the positive effect of AA on root growth which accordingly increasing absorption of nitrate. (El-Bassiouny *et al.*, 2005) indicated that foliar spray with AA on faba bean increased total carbohydrates, protein, K, P and Ca contents in seeds. (Khafaga *et al.*, 2014) found that AA foliar application increased N, P and K contents in seeds of faba bean.

### 5.3. Effect of the interaction between bio-fertilizer (*Rhizobium*) and antioxidants:

As for the effect of inoculating pea seeds with *Rhizobium* along with foliar spray with antioxidants on seeds bioconstituents i.e., N, P, K, crude protein, total carbohydrates, ascorbic and salicylic acids %, results indicate that inoculation along with foliar spray with combination of SA and AA at 250 mg/liter of each was the most effective treatment which gave the highest values followed by *Rhizobium*+SA and *Rhizobium*+AA treatments, respectively during both seasons. In this respect, increasing of pea seeds biochemical compositions with different applied treatments consider as a direct result of increasing photosynthesis rate and efficiency. That was preceded with large photosynthetic area, **Table (2)** as well as high concentration of photosynthetic pigments, **Table (4)**. Such promotional effect of applied antioxidants could be due to their similar effects on leaves, surfaces of photoassimilation. Thereby, the capacity of CO<sub>2</sub> fixation and carbohydrates synthesis. Treated plants with these treatments were of higher carbohydrates and sugars concentrations might be exported sufficient sugars at early stage, those essentially required for protective effect on the most sensitive reproductive organs i.e., pollen grains and ovules and their viability and in turn, increasing of the efficiency of fertilization process as well as pods setting activities. Such accumulation in osmolytes is necessary for the maintenance of plants under saline stress due to its important roles in osmoregulation and osmotic adjustment, the disturbance in plant osmotic under stress conditions could attributed to the imbalance of most metabolic processes, i.e., photosynthesis, respiration, transpiration, enzyme activities and protein synthesis. Generally, it could be concluded that different applied treatments were mostly effective, which induced an active metabolically case and the most effective internal defensive antioxidant mechanism i.e., carotenoids, phenols, sugars, protein and so on. At the same time, this was accompanied with good morphological, minerals status and agronomical performances. The

results are coincided with those of (Chame *et al.*, 2016) indicated that inoculation bean seeds with

biofertilizer and using SA reduced the effect of salinity on seed protein content.

**Table 8.** N, P, K and crude protein % of pea seeds as affected by *Rhizobium*, antioxidants and their interactions during 2015/2016 and 2016/2017 growing seasons.

Treatments	2015/2016					2016/2017				
	Control	SA	AA	SA + AA	mean	Control	SA	AA	SA + AA	mean
Nitrogen										
- <i>Rhizobium</i>	2.73 e	3.00 d	3.23 cd	3.22 cd	3.04 b	2.75 e	3.07 d	3.38 c	3.28 cd	3.12 b
+ <i>Rhizobium</i>	3.41 bc	3.49 ab	3.72 a	3.69 a	3.58 a	3.52 bc	3.65 ab	3.75 ab	3.86 a	3.69 a
mean	3.07 c	3.25 b	3.47 a	3.45 a		3.13 c	3.36 b	3.56 a	3.57 a	
Phosphorus										
- <i>Rhizobium</i>	0.25 d	0.29 c	0.31 c	0.31 c	0.29 b	0.28 e	0.30 d	0.31 d	0.32 d	0.30 b
+ <i>Rhizobium</i>	0.34 b	0.34 b	0.36 ab	0.36 a	0.35 a	0.34 c	0.35 bc	0.37 ab	0.38 a	0.36 a
mean	0.29 c	0.32 b	0.33 a	0.34 a		0.31 c	0.33 b	0.34 ab	0.35 a	
Potassium										
- <i>Rhizobium</i>	1.91 d	2.18 cd	2.29 c	2.25 c	2.16 b	2.10 f	2.37 e	2.40 de	2.40 de	2.32 b
+ <i>Rhizobium</i>	2.45 bc	2.66 ab	2.80 a	2.87 a	2.69 a	2.63 cd	2.79 bc	3.07 a	2.99 ab	2.87 a
mean	2.18 b	2.42 a	2.54 a	2.56 a		2.36 b	2.58 a	2.73 a	2.70 a	
Crude protein										
- <i>Rhizobium</i>	17.06 e	18.78 d	20.21 cd	20.12 cd	19.04 b	17.19 e	19.18 d	21.12 c	20.50 cd	19.50 b
+ <i>Rhizobium</i>	21.31 bc	21.84 ab	23.25 a	23.09 a	22.37 a	22.00 bc	22.84 ab	23.43 ab	24.12 a	23.10 a
mean	19.18 c	20.31 b	21.73 a	21.61 a		19.59 c	21.01 b	22.28 a	22.31 a	

Values followed by same letters within a column not significantly differed at 5% according to Duncan's multiple range test. SA: Salicylic acid at 250 mg/L. and AA: Ascorbic acid at 250 mg/L.

**Table 9.** Total carbohydrates, ascorbic and salicylic acids % of pea seeds as affected by *Rhizobium*, antioxidants and their interactions during 2015/2016 and 2016/2017 growing seasons.

Treatments	2015/2016					2016/2017				
	Control	SA	AA	SA + AA	mean	Control	SA	AA	SA + AA	mean
Total carbohydrates										
- <i>Rhizobium</i>	45.05 e	48.16 d	49.19 cd	49.02 cd	47.85 b	46.24 d	48.34 cd	50.07 bc	49.31 bc	48.49 a
+ <i>Rhizobium</i>	50.24 bc	50.99 ab	52.02 a	51.99 a	51.31 a	50.28 bc	51.32 ab	52.68 a	52.83 a	51.78 a
mean	47.64 b	49.57 a	50.60 a	50.50 a		48.26 b	49.83 ab	51.37 a	51.07 a	
Ascorbic acid										
- <i>Rhizobium</i>	3.46 e	6.31 d	8.04 c	8.99 b	6.70 b	4.82 d	7.75 c	10.68 b	10.46 b	8.42 b
+ <i>Rhizobium</i>	3.50 e	6.39 d	11.05 a	10.85 a	7.95 a	5.01 d	7.83 c	12.28 a	12.20 a	9.33 a
mean	3.48 d	6.35 c	9.55 b	9.92 a		4.91 c	7.79 b	11.48 a	11.33 a	
Salicylic acid										
- <i>Rhizobium</i>	9.38 d	13.97 b	12.21 c	13.98 b	12.38 b	10.87 d	15.31 b	12.57 c	15.45 b	13.55 b
+ <i>Rhizobium</i>	9.40 d	16.85 a	12.30 c	16.78 a	13.83 a	10.88 d	18.16 a	12.71 c	18.25 a	15.00 a
mean	9.39 c	15.41 a	12.25 b	15.38 a		10.87 c	16.73 a	12.64 b	16.85 a	

Values followed by same letters within a column not significantly differed at 5% according to Duncan's multiple range test. SA: Salicylic acid at 250 mg/L. and AA: Ascorbic acid at 250 mg/L.

## Conclusion

Results obtained in the present study confirmed that inoculation pea (*Pisum sativum* L.) seeds with *Rhizobium*, foliar spray with antioxidants i.e., salicylic acid (SA), ascorbic acid (AA) and SA+AA at 250 mg L<sup>-1</sup> of each as well as their interactions were able to produce positive effects in pea plant. In particular, improving fresh and dry biomasses,

photosynthetic pigment contents, yield components as well as seed bioconstituents have been observed due to treatments application under salinity conditions. Consequently, supply of inoculation pea seeds with *Rhizobium* along with foliar spray of SA+AA was the most effective treatment on alleviating the negative effects of saline stress.



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تأثير التلقيح بالريزوبيوم والرش الورقي بأحماض الساليسيليك والإسكوريك على النمو والمحصول وجودة البذور في نبات البسلة المنزرع في أرض متأثرة بالملوحة ، الوادي الجديد- مصر

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أجريت هذه الدراسة بهدف دراسة تأثير معاملة البذور بالتسميد الحيوي (الريزوبيوم) والرش الورقي بمضادات الأكسدة (حامض الساليسيليك ، حامض الإسكوريك ، حامض الساليسيليك + حامض الإسكوريك بتركيز ٢٥٠ ملجم/ لتر لكل منهما) والتفاعل بينهم على صفات النمو ، المحصول ومكوناته والمكونات الكيميائية للبذور في نبات البسلة صنف ماستر بي المنزرع في أرض متأثرة بالملوحة ، الوادي الجديد - مصر خلال موسمي الزراعة ٢٠١٦/٢٠١٥ و ٢٠١٧/٢٠١٦ لتعظيم الإنتاجية تحت ظروف الإجهاد الملحي. بالنسبة للمعاملة بالريزوبيوم تم تلقيح البذور قبل الزراعة بالبكتريا المثبتة للنيتروجين (الريزوبيوم) بينما تم الرش الورقي بمعاملات مضادات الأكسدة بعد ٢٠ يوم من الزراعة مع التكرار ٣ مرات بفواصل زمني ١٠ أيام خلال موسمي النمو.

سجلت القياسات الأتية خلال فترة النمو وعند الحصاد: صفات النمو الخضري ، عدد العقد الجذرية ، الوزن الطازج والجاف للعقدة الجذرية ، محتوى الأوراق من صبغات البناء الضوئي ، المحصول الطازج والجاف ومكوناته بالإضافة إلى المكونات الكيميائية للبذور.

أظهرت النتائج المتحصل عليها أن تلقيح البذور بالريزوبيوم أدى إلى زيادة معنوية في كل الصفات المدروسة مقارنة بالبذور الغير معاملة بالريزوبيوم (الكنترول).

أدى الرش الورقي للنبات بمعاملات مضادات الأكسدة (حامض الساليسيليك وحامض الإسكوريك بتركيز ٢٥٠ ملجم/ لتر لكل منهما بالإضافة إلى معاملة الخلط بينهما) إلى زيادة معنوية في الصفات المدروسة مقارنة بالنبات الغير معاملة (الكنترول) وكانت أفضل المعاملات في هذا الصدد التداخل بين الساليسيليك والإسكوريك بتركيز ٢٥٠ ملجم/ لتر لكل منهما.

أدت معاملات التفاعل بين تلقيح البذور بالريزوبيوم والرش الورقي بمضادات الأكسدة (حامض الساليسيليك وحامض الإسكوريك بتركيز ٢٥٠ ملجم/ لتر لكل منهما بالإضافة إلى معاملة الخلط بينهما) إلى زيادة معنوية في صفات النمو الخضري ، محتوى الأوراق من صبغات البناء الضوئي ، عدد ووزن العقد البكتيرية ، المحصول الطازج والجاف ومكوناته ، محتوى البذرة من النيتروجين ، القوسفور ، البوتاسيوم ، البروتين الكلي ، الكربوهيدرات الكلية ، أحماض الساليسيليك والإسكوريك وكانت أفضل المعاملات في هذا الصدد معاملة البذور بالريزوبيوم مع الرش الورقي بحامض الساليسيليك وحامض الإسكوريك معاً مقارنة بالمعاملات الأخرى.