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EVALUATION OF SOME CHICKPEA GENOTYPES TO BACTERIAL INOCULATION

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ABSTRACT: Field experiment was carried out during the two successive seasons (2015-2016 and 2016-2017) at Sids Research Station, Agricultural Research Center (ARC), Bani Swif Governorate, Egypt, to evaluate the response of some new chickpea genotypes cultivated under Egyptian soil conditions for rhizobial inoculation alone or in combination with foliar inoculation of Pink Pigmented Facultatively Methylotrophic bacteria (PPFMs) as Plant Growth Promoting Rhizobacteria (PGPR). Nodulation status, some vegetative growth and yield parameters were determined. The obtained results cleared that all chickpea genotypes tested were positively responded to the native soil rhizobia and formed root nodules. Rhizobial inoculation alone or in combination with foliar application of PPFMs bacteria (5 L fed^{-1}) scored significant increases in nodule numbers, plant dry weight, yield per plant as well as seed yield at the both seasons as compared to untreated treatments. Using rhizobial inoculation and PPFMs bacteria emphasized the superiority and gave of the highest values at all tested parameters. Generally, the second season gave the highest values at all plant tested parameters as compared to uninoculated ones. Chickpea genotypes GT3, GT4 and GT7 emphasized higher response to cultivated under Egyptian soil conditions and gave higher values for nodules number and dry weight, growth parameters i.e. plant dry weight and plant N-content and yield parameters i.e. yield per plant, seed index and seed yield ton. fed^{-1} as compared to chickpea variety G195.

Key words: Chickpea genotypes, Rhizobial inoculation, Pink- Pigmented Facultatively Methylotrophic bacteria (PPFMs), Foliar application.

INTRODUCTION

Legumes-*Rhizobium* symbiosis is undoubtedly the most important N_2-fixing process and play a subtle role in providing nitrogen and maintaining/improving soil fertility. Symbiosis between legumes and rhizobia are of a considerable environmental and agricultural importance, since they are responsible for most of the atmospheric nitrogen fixed on land **(Graham and Vance. 2003)**.

Chickpea (*Cicer arietinum* L.) is one of the earliest grain crops cultivated by man and has been found in Middle Eastern archeological sites dated at 7500–6800 B.C., **(Williams and Singh. 1987).** Chickpea is highly self-pollinating with an out crossing rate of less than 1%. Two main types of chickpea cultivars are grown globally, representing two diverse sub gene pools: Kabuli and Desi. The Kabuli types are generally grown in the Mediterranean region, southern Europe, western Asia, and northern Africa and the Desi types are grown mainly in Ethiopia and the Indian subcontinent. In spite of the above-mentioned constraints, extensive international breeding efforts have led to the development of over 300 improved varieties **(Gowda and Gaur .2004).**

Chickpea is grown in about 50 countries, it can fix up to 140 kg nitrogen ha^{-1} and meet up to 80% of its nitrogen requirement from symbiotic nitrogen fixation **(Abo Taleb (1998)** and **Al-hudaiji (2015)**). Chickpea has the highest nutritional compositions and rich in fiber and minerals (phosphorus, calcium, magnesium, iron, and zinc). Its lipid fraction is high in unsaturated fatty acids in addition to having high protein content (20–22%). **(Zohary and Hopf. 2000)**. **Singh *et al.*, (2008)** illustrated the genetic relationships between the cultivated chickpea and its wild relatives is a prerequisite to track the evolution of cultivated species and also to determine the close relatives which can be exploited for introgression of useful traits into the cultigen in plant breeding programs and many developing countries are substantial research programs to improve its yield, disease resistance and nutritional quality.

Vessey (2003) reported that numerous species of soil bacteria, which flourish in the rhizosphere of plants, but which may grow in, on, or around plant tissues, stimulate plant growth by various mechanisms. These bacteria are correctively known as Plant Growth Promoting Rhizobacteria (PGPR). The search for PGPR and investigation of their modes of action are increasing to exploit them commercially as biofertilizer. The mode of action of the biofertilizers includes fixing nitrogen, increasing the availability of nutrients in the rhizosphere, positively influencing both morphology and growth of roots, and promoting other beneficial plant-microbe symbiosis. The combination of these modes of actions in PGPR is also addressed.

Madhiayan *et al*. (2005) reported that The genus *Methylobacterium*- as PGPR - includes a variety of pink pigmented facultative methylotrophic bacteria (PPFMs) that promote plant growth by generating vitamins ,phytohormones (IAA, gibberellins and cytokinins) ,as well as supply nitrogen to plant through diazotrophy and indirectly reduce or prevent the deleterious effects of pathogenic microorganisms, through induced systemic resistance**.**

Etesami and Maheshwari (2018) stated that, combined use of PGPRs in agricultural environments may be a suitable approach to sustainably integrate with chemical fertilizers and lead to plant health improvements that play an important role in reducing the amount of chemicals to achieve sustainable agricultural productivity.

The present work aims to evaluate new chickpea genotypes response to rhizobial inoculation alone or in combination with application of PPFMs bacteria as PGPR bacterial inoculation and its role in enhancing the vegetative growth, seed yield and yield quality of chickpea plants under Egyptian soil conditions.

MATERIAL AND METHODS

1. Soil used

A field experiment was layout during the two successive seasons (2015-2016 and 2016-2017) at Sids Research Station, Bani Swif Governorate, Agricultural Research Center (ARC). Physico-chemical properties of the used soil was carried out according to **Jackson (1973)** at soil analysis Lab., Soils, Water and Environment Research Institute (SWERI), ARC, Giza, and is shown in Table (1).

Table 1. Some physico-chemical properties of used soil

Property	Values
Mechanical analysis	
Sand	19.5
Silt	34.0
Clay	64.5
Texture grand	Clay loam
Physical analysis	
S. P. %	48.77
PH	7.72
E.C. dSm	1.04
Organic Carbon %	0.53
Organic Matter %	0.91
Soluble Nitrogen %	62.46
Total Nitrogen %	0.028
Chemical analysis	
Available _P %	7.62
Available _K %	311.60
EDTA_ extractable	
Fe	8.60
Mn	4.31
Zn	4.10
Cu	1.81
Soluble Cations (meql^{-l})	
Ca^{++}	3.00
Mg^{++}	1.36
Na^{+}	5.12
K^{+}	0.98
Soluble Anions (meql^{-l})	
CO_3^{--}	0.00
HCO_3^{-}	1.51
Cl^{-}	1.72
SO_4^{--}	7.23

2. Seeds used

Seeds of Chickpea (*Cicer arietinum* L.) variety Giza 195 (G195),and seven genotypes namely: GT1 (FLP0893C), GT2 (S091013), GT3 (S090642), GT4 (FLP0846C), GT5 (FLP0872), GT6 (FLP0847C) and GT7 **(**FLIP08-141C) were used in field experiment at rate 35 kg.fed^{-1} and kindly supplied by Legume Crop Research Dept. Field Crop Research Institute, (ARC), Giza, Egypt.

3. Bacterial strains used

3.1. Two strains of *Mesorhizobium ciceri* namely ICARDA 36 and NIFTAL 1148 specific to Chickpea grown on Yeast extract Mannitol agar (YEM) medium **(Vincent, 1970)** were used as mixture basal peat inoculant at rate 4g inoculant to 100 g seeds at the time of planting as seed coating method according to **Abo Taleb (1998).**

3.2. Two strains of PPFMs bacteria namely *Methylobacterium mesophilicum* and

Methylobacterium radiotolerans grown on Methanol Mineral Salts (MMS) agar medium **(Holland and Polacco, 1992)** were used as foliar inoculation at rate of 5 L.Fed^{-1} **(Shehata, Sawsan *et al*., 2006)** after 30 days of planting .These strains were kindly obtained from Biofertilizers Production Unit, Agricultural Microbiology Dept., Soils, Water and Environment Research Institute, ARC, Giza, Egypt.

4. Fertilizers used

The recommended doses of P and K fertilizers: 100 Kg superphosphate (15.5 % P_2O_5 fed^{1}) and 50 Kg potassium sulphate (24 K_2O fed^{-1}) were added during field experiment preparation. N-fertilization as ammonium sulphate (20.5 % N) was applied at 15 and 50 Kg N fed^{-1} and were added at 15 ,21 and 35 days after planting.

5. Treatments

Three treatments with 3 replications were allocated in a completely randomized block design as follows:

1. Un-inoculated plants + 50 Kg N fed^{-1}.
2. Rhizobial inoculation +15 Kg N fed $^{-1}$.
3. Rhizobial inoculation+ foliar application with PPFMs (5 L.fed $^{-1}$, at 30 days after planting) + 15 Kg N fed^{-1}.

The plot area was 3x 3.5 m^{2}.

6. Determinations

6.1. Growth stage: Samples were taken after 75 days of planting to determine: nodulation status (number and dry weight of nodules) according to **Vencent (1970)** and some vegetative growth parameters (plant dry weight and plant nitrogen content).

6.2. Harvest stage: number of branches, number of pods, seed yield (kg. plot^{-1} and ton. fed^{-1}), yield of plant and seed index were determined in samples after 140 days of planting, according to **A.O.A.C. (1990).**

7. Statistical analysis

Data were subjected to an analysis of variance (ANOVA) and the least significant difference test (LSD) at P <0.05, by using (MSTAT) Program according to **Snedecor and Cochran (1980).**

RESULTS AND DISCUSSION

I. Growth stage

1. Nodulation status

1.1. Nodule number (nod. no. plant^{-1})

Data in table (2) show that, the all chickpea genotypes responded to the native rhizobia and formed root nodules bacteria but scored the lowest values of nodules formation in both seasons which ranged from 11 to 22 (nod. no. plant^{-1}) as compared to other tested treatments. Inoculation with specific rhizobia scored significant increases in both seasons and such increases ranged between 22.7 -254.5% as compared to un- inoculated ones. Inoculation with specific rhizobia in combination with PPFMs as foliar application scored highest value (53 nod. no. plant^{-1}) and led to gave significant increases in number of nodules ranged from 8.6-34.4% in both seasons as compared to the treatments which received rhizobial inoculation.

1.2. Nodule dry weight (mg plant^{-1})

Data in Table (3) reveal that, the un-inoculated treatment recorded the lowest value of nodules dry weight (30 mg plant^{-1}) in both seasons as compared to other treatments. Inoculation with specific *Mesorhizobium* scored higher values (68-104 mg plant^{-1}) as compared to un-inoculated treatment in both seasons. Application PPFMs as foliar spraying in the presence of rhizobial inoculation scored highest significant increases and recorded nodules dry weight up to (163 mg plant^{-1}) as compared to inoculation with specific *Mesorhizobium* alone. Inoculated chickpea genotypes GT4 having the highest value in nodule dry weight (up to 104 mg plant^{-1}) among all inoculated chickpea genotypes as compared to uninoculated treatments.

The above mentioned data are in agreement with the observations made by **Joshi *et al*. (2000), Stougaard (2000), Begum *et al*. (2001)** and **Ogutcu *et al*. (2008)** who reported that PPFMs plays very important role in root nodule initiation, development and function of many legume plants ,*i.e.* alfa alfa and soybean and the number and weight of nodules per plant showed significant response to nitrogen fertilization rates , inoculation with specific rhizobial strains and chickpea varieties. Also, **Gopalakrishnan *et al*. (2018)** reported that, rhizobial inoculation as such or in-combination with PGPR enhanced the nodule number, nodule weight and shoot weight over the un-inoculated control of chickpea cultivars.

Table 2. Number of nodules and percentage of increases for various chickpea genotypes as affected by application of different bacterial inoculation at 75 days after planting

Parameters / Genotypes	Number of nodules (no. nod. plant^{-1})										
	Un inoculated			R. inoculation			% increases*	R.+ ppfm inoculation			% increase**
	S1	S2	x̄	S1	S2	x̄		S1	S2	x̄	
GT1	12	16	14	25	36	31	121.4	26	49	38	22.6
GT2	25	19	22	16	38	27	22.7	38	53	46	70.3
GT3	14	22	18	19	42	31	72.2	22	45	34	9.7
GT4	10	12	11	40	38	39	254.5	38	53	46	17.9
GT5	28	16	21	36	44	40	90.4	47	51	49	18.4
GT6	18	13	16	26	43	35	118.8	27	49	38	8.6
GT7	15	13	14	25	39	32	128.6	31	55	43	34.4
G195	23	21	22	41	47	44	100	48	57	53	20.5
L.S.D	6.55	7.31		8.62	5.72			5.53	3.78		

S: season **GT**: Genotype
* % of increases as compared to un-inoculated treatment.
** % of increases as compared to rhizobial inoculation treatment.

Table 3. Dry weight of nodules for various chickpea genotypes as affected by application of different bacterial inoculation at 75 days after planting

Parameters / Genotypes	Dry weight of nodules (mg plant^{-1})								
	Un inoculated			R. inoculation			R.+ ppfm inoculation		
	S1	S2	x̄	S1	S2	x̄	S1	S2	x̄
GT1	31	43	36	65	98	82	70	139	105
GT2	86	67	77	40	95	68	106	219	163
GT3	32	55	44	50	103	77	52	116	84
GT4	24	35	30	110	97	104	110	128	119
GT5	77	42	60	91	115	103	142	110	126
GT6	61	45	53	71	107	89	72	98	85
GT7	35	28	32	59	82	71	88	113	101
G195	41	39	40	88	103	96	153	167	160
L.S.D	15.70	11.40		17.8	9.31		27.11	30.75	

S: season **GT**: Genotype

2. Vegetative growth

2.1. Plant dry weight (g plant^{-1})

Data in table (4) show that, the high significant differences in plant dry weight were evident among the all tested treatments. Un-inoculated chickpea genotypes recorded the lowest plant dry weight ranged 1.5 to 2.9 g plant^{-1}. Inoculated plants scored higher values ranged from 2.5 to 3.1 g plant $^{-1}$, significant increases ranged from 11.5 to 80 % of plant dry weight as compared to uninoculated treatments. Rhizobial inoculation in-combination with foliar PPFMs bacteria gave the highest plant dry weight (2.9 - 3.3 g plant^{-1}) and recorded significant increases up to 11.1% were observed as compared to inoculated treatments as such. Chickpea genotypes GT3, GT4 and GT7 having the highest plant dry weight as compared to other chickpea genotypes tested among the two tested seasons.

These results are in harmony with **Peix *et al*. (2001)** and **Shukla *et al*. (2012)** who found a positive response of chickpea genotypes to inoculation with rhizobia and /or PPFM bacteria and recorded significant increases in plant dry biomass accumulation compared to uninoculated ones. Generally, the increase in dry matter accumulation due to seed inoculation with rhizobia and PPFM s indicates the favorable response of chickpea genotypes to inoculation **(Orf, Heba *et al*. 2014).**

Table 4. Plant dry weight for various chickpea genotypes as affected by application of different bacterial inoculation at 75 days after planting

Parameters / Genotypes	Plant dry weight (g plant^{-1})										
	Un inoculated			R. inoculation				R.+ ppfm inoculation			
	S1	S2	x̄	S1	S2	x̄	% increase*	S1	S2	x̄	% increase**
GT1	1.4	1.6	1.5	2.6	2.7	2.7	80.00	2.7	2.9	2.8	3.6
GT2	1.8	1.9	1.9	2.4	2.6	2.5	31.60	2.6	2.7	2.7	8.0
GT3	2.6	2.8	2.7	2.5	2.9	2.7	0.00	2.8	3.1	3.0	11.1
GT4	2.6	3.1	2.9	2.4	3.1	2.8	0.00	3.0	3.2	3.1	10.7
GT5	1.5	2.1	1.8	2.4	2.9	2.7	50.00	2.8	3.0	2.9	7.4
GT6	1.9	2.7	2.3	2.7	3.2	3.0	30.43	2.8	2.9	2.9	0.0
GT7	2.3	2.8	2.6	2.6	3.1	2.9	11.50	3.1	3.3	3.2	10.3
G195	2.4	2.6	2.5	2.9	3.2	3.1	24.00	3.2	3.4	3.3	6.5
L.S.D	0.57	0.63	----	n.s	0.11	----		0.32	0.28	----	

S: season **GT**: Genotype
*% of increases as compared to un-inoculated treatment.
% of increases compared to rhizobial inoculation treatment.**

2.2. Plant N-content (mg plant^{-1})

Data in Table (5) reveal that, un-inoculated treatment recorded the lowest values of plant N-content, such values ranged from 41.7 to 74.2 mg plant^{-1} as compared to other treatments among the two tested seasons. Inoculation with specific *Mesorhizobium* scored higher values ranged from 87.4 to 94.8 mg plant^{-1}. The highest values of plant N-content were found at treatments which received rhizobial inoculation in combination with PPFMs foliar application with chickpea genotypes GT3, GT4 and GT7 as well as chickpea variety G195.

Table 5. Plant N-content for various chickpea genotypes as affected by application of different bacterial inoculation at 75 days after planting

Parameters / Genotypes	Plant N-content (mg plant^{-1})								
	Un inoculated			R. inoculation			R. + ppfm inoculation		
	S1	S2	x̄	S1	S2	x̄	S1	S2	x̄
GT1	37.6	45.8	41.7	84.9	93.2	89.1	56.7	67.2	61.9
GT2	53.9	56.9	55.4	74.1	82.7	78.4	64.6	75.7	70.2
GT3	57.9	63.2	60.1	61.4	96.4	78.9	72.6	81.8	77.2
GT4	70.2	78.1	74.2	61.9	82.8	72.4	70.1	89.2	79.7
GT5	49.6	61.7	55.7	78.4	94.7	86.6	96.7	99.3	98.0
GT6	48.9	63.9	56.4	74.2	115.3	94.8	94.3	106.2	100.3
GT7	44.9	53.2	49.1	76.4	98.3	87.4	97.5	121.7	109.6
G195	68.4	71.3	69.9	82.4	105.6	94.0	112.5	134.8	123.7
L.S.D	27.1	22.7	----	11.3	16.7	----	27.3	35.4	----

S: season **GT:** Genotype

The obtained data are in agreement to **Polacco and Holland (1993)** who reported that, application of inoculation with PPFMs resulted increasing of plant dry weight of soybean plants as compared to untreated ones. **Holland (1997)** reported that the activities of PPFMs could make a biochemically measurable and physiologically meaning full contribution to plant nitrogen accumulation and metabolism. The mentioned data are in harmony with **Yates *et al.* (2007)** who reported that PPFM bacteria play very important role in plant nitrogen content, and symbiotically benefit the plant species. Also, **Sharma *et al*. (2016)** stated that inoculated peanut seedlings with rhizobia as such or in-combination with PGPR scored significant increase in total nitrogen (N) content (up to 76%) over the non-inoculated control.

3. Harvest stage

3.1. Number of branches (No. Plant^{-1})

Application of rhizobial inoculation alone or in-combination with PPFMs bacteria led to gave higher values and scored significant increases at number of branches at various tested chickpea genotypes as shown in Table (6) .Values of number of branches ranged from 6.0 to 12.6, 8.3 to 13.3 and 10.2 to 13.7 for untreated chickpea genotypes, inoculated plants and rhizobial inoculation in-combination with PPFMs bacteria respectively through the tested seasons. Genotype GT4 recorded the highest value of number of branches per plant and value was up to13.2 among the two tested seasons at various treatments and followed by GT7 chickpea genotypes (13.1).In this respect, **Rudresh *et al.* (2005)** studied the effect of inoculation with Rhizobium on growth attributes and observed that chickpea gave higher plant height (3.3%), number of branches per plant (23.3%) and biomass per plant (144%) as compared to uninoculated control. In similar findings, **Elkoca *et al.* (2008)** revealed that rhizobial inoculation increased plant height, shoot dry weight and chlorophyll content in chickpea. These findings are in agreement with that of **Giri and Joshi (2010)**.

Table 6. Number of branches for various chickpea genotypes as affected by application of different bacterial inoculation at harvest stage

Parameters / Genotypes	Number of branches (No Plant^{-1})								
	Un inoculated			R. inoculation			R.+ ppfm inoculation		
	S1	S2	$\bar{x}$	S1	S2	$\bar{x}$	S1	S2	$\bar{x}$
GT1	6.0	8.3	7.2	11.6	13.3	12.5	12.7	13.3	13.0
GT2	7.6	8.5	8.6	10.0	13.3	11.7	11.6	13.3	12.5
GT3	8.0	11.0	9.5	10.0	12.6	11.3	10.6	12.6	11.6
GT4	9.6	11.6	10.6	11.6	12.6	12.1	12.8	13.5	13.2
GT5	9.5	12.6	11.1	8.3	12.6	10.5	10.2	13.5	11.9
GT6	11.0	11.4	11.2	11.6	11.5	11.6	11.8	12.5	12.2
GT7	8.3	9.6	8.9	11.6	11.3	11.5	12.5	13.7	13.1
G195	8.3	11.0	9.7	10.3	11.6	11.0	10.8	12.5	11.7
L.S.D	2.55	2.11	----	1.63	0.81	-----	1.17	1.08	----

S: season **GT**: Genotype

3.2. Number of pods (No. Plant^{-1})

Data in Table (7) show that, un -inoculated treatment gave the lowest number of pods at all tested chickpea genotypes as compared to other treated treatments in the both two seasons, these values ranged from (21.2 to 39.9 No. plant^{-1}). Inoculation with specific *Rhizobium* as such or in combination with PPFM bacteria emphasized the superiority in number of pods and recorded values ranged from 33.3 to 69.3 and 35.4 to 72.4 for inoculated plants as such and rhizobial inoculation in-combination with PPFMs bacteria respectively as an average of the two tested seasons.

Table 7. Number of pods of various chickpea genotypes as affected by application of different bacterial inoculation at harvest stage

Parameters / Genotypes	Number of pods (No. Plant^{-1})								
	Un inoculated			R. inoculation			R.+ ppfm inoculation		
	S1	S2	$\bar{x}$	S1	S2	$\bar{x}$	S1	S2	$\bar{x}$
GT1	21.6	30.0	25.8	20.6	46.0	33.3	22.5	48.2	35.4
GT2	22.6	28.3	25.5	59.6	69.0	64.3	58.9	72.1	65.5
GT3	24.6	33.3	29.0	41.6	35.6	38.3	45.3	51.5	48.4
GT4	21.6	24.6	23.1	44.3	35.6	40.0	44.5	55.3	49.9
GT5	20.0	22.3	21.2	33.3	35.6	34.5	47.2	57.3	52.3
GT6	36.6	43.3	39.9	42.3	59.0	50.7	46.7	59.2	53.0
GT7	26.6	27.0	26.8	63.0	75.6	69.3	67.5	77.2	72.4
G195	26.3	26.6	26.5	40.3	44.3	42.3	50.3	54.3	52.3
L.S.D	5.43	6.17	----	21.17	15.81	7.38	11.7	8.92	-----

S: season **GT:** Genotype

These data were in harmony with those obtained from **Sharar *et al*. (2000)** and **Khan *et al*. (2003)** who reported that, Number of pods per plant and number of seeds per plant were reported to be 21.8% and 10.5% higher, respectively in chickpea inoculated with *Rhizobium* over uninoculated control. Similar observations reported in other studies where inoculation of chickpea with rhizobia increased plant growth, ground dry matter, number of pods, seed yield, and nitrogen fixation under various climatic conditions **(Fatima *et al*., 2008).**

3. Yield parameters

3.1. Yield per plant (g plant $^{-1}$)

Untreated chickpea genotypes recorded lower values for yield per plants and these values ranged from 9.9 to 14.2 g plant $^{-1}$ as shown in Table (8). Application of rhizobial inoculation as such did support plant yield and led to gave higher values and scored significant increases ranged from 27.5 to 175 %, as compared to un-inoculated treatments. On the other hand, rhizobial inoculation in-combination with PPFMs bacteria having the highest plant yield values among the all tested treatments in the both seasons and gave percentage increases ranged from 6.4 to 59.4 % as compared to inoculated chickpea genotypes as such.

Table 8. Yield of plant of various chickpea genotypes as affected by application of different bacterial inoculation at harvest stage

Parameters / Genotypes	Yield per plant (g plant^{-1})										
	Un inoculated			R. inoculation				R.+ ppfm inoculation			
	S1	S2	$\bar{x}$	S1	S2	$\bar{x}$	% increase*	S1	S2	$\bar{x}$	% increase**
GT1	8.3	11.6	9.9	24.4	28.3	26.4	166.7	27.5	28.6	28.1	6.4
GT2	10.0	16.6	13.3	27.1	42.6	34.9	162.4	29.7	52.3	41.0	17.5
GT3	13.3	15.0	14.2	12.9	23.3	18.1	27.5	24.7	30.1	27.4	51.4
GT4	11.6	13.3	12.5	15.3	23.3	19.3	54.4	28.2	30.2	29.2	51.3
GT5	10.0	11.6	10.8	16.9	18.1	17.5	62.0	26.9	28.9	27.9	59.4
GT6	8.3	15.0	11.7	18.3	25.4	21.9	87.2	30.5	33.6	32.1	46.6
GT7	8.3	14.0	11.2	25.6	36.0	30.8	175.0	32.7	39.2	36.0	16.9
G195	9.6	16.6	13.1	23.3	27.8	25.6	95.4	29.3	32.9	31.3	22.3
L.S.D	2.17	1.21	-----	6.33	9.81	-----		2.75	39.7	----	

S: season **GT:** Genotype
% of increases *as compared to un-inoculated treatment.
% of increase **as compared to rhizobial inoculation treatment.

These data are in agreement with those of **Suresh Reddy *et al.* (2002)** who worked on the effect of combined inoculation of PPFMs and *Rhizobium* on groundnut cultivar Co (Gn) 4 and observed that, there was significant increase in plant growth, biomass production and yield parameters of groundnut. **Radha *et al*. (2009)** also reported that, inoculation of *Methylobacterium* isolates incombination with *Bradyrhizobium japonicum* strain SB120 had significant influence on different plant growth parameters, nutrient uptake and yield of soybean plants.

3.2. Seed index (g 100 seed^{-1})

Data in Table (9) cleared that, both rhizobial inoculations as such or in-combination with PPFMs bacteria gave higher values for seed index at all tested chickpea genotypes as compared to un treated treatments. GT4 and GT7 chickpea genotypes as well as chickpea variety G195 recorded the highest values of seed index and these values were 36.9, 33.6 and 34.6 (g 100seed^{-1}) for GT4, GT7 and G195 respectively among the two tested seasons. The above mentioned data are in harmony with those obtained by **Sharar *et al*. (2000)** who reported that number of seeds per plant scored 10.5% higher in chickpea inoculated with *Rhizobium* over uninoculated control. Further, **Khan *et al*. (2003)** and **Ali *et al*. (2014)** revealed that 1000-seed weight was significantly better with inoculation in chickpea. These findings are in agreement with that of **Elkoca *et al*. (2008)** , **Akhtar and Siddiqui (2009)** and **Meena *et al*. (2013)** who reported that the performance of the chickpea plants was better in inoculation treatments in comparison to control .Two years of trials under field conditions also showed that bacterial inoculations significantly affected all parameters investigated, compared with control.

Table 9. Seed index of various chickpea genotypes as affected by application of different bacterial inoculation at harvest stage.

Parameters / Genotypes	Seed index (g 100 seed^{-1})								
	Un inoculated			R. inoculation			R.+ ppfm inoculation		
	S1	S2	x̄	S1	S2	x̄	S1	S2	x̄
GT1	26.4	28.4	27.4	27.0	27.2	27.1	28.2	31.5	29.9
GT2	28.5	30.5	29.5	29.5	31.8	30.7	32.5	32.6	32.6
GT3	26.5	26.8	26.7	30.2	30.9	30.6	31.7	34.8	33.3
GT4	26.9	26.5	26.7	29.9	30.1	30.0	36.3	37.5	36.9
GT5	26.3	25.7	26.0	30.0	32.8	31.4	33.6	37.5	36.9
GT6	26.6	27.4	27.0	29.6	32.7	31.2	32.6	35.1	33.9
GT7	29.0	27.5	28.3	29.2	31.2	30.2	33.2	33.7	33.6
G195	28.9	29.5	29.2	29.5	32.6	31.1	33.5	35.7	34.6
L.S.D	1.77	2.34	----	1.93	2.11	----	1.82	1.73	----

S: season **GT:** Genotype

3.3. Seed yield (kg plot^{-1})

Application of various bacterial treatments led to enhance seed yield (kg plot^{-1}) and recorded higher values as compared to untreated treatments which recorded the lowest values for seed yield (kg plot^{-1}) as shown in Table (10). GT7, GT3, GT4 chickpea genotypes and variety G195 were responded to rhizobial inoculation in-combination with PPFMs bacteria and scored the highest seed yield (kg plot^{-1}) and these values were 2.13,1.99,1.87 and 2.40 for GT7, GT3, GT4 and chickpea genotypes and G195 respectively. In this respect, **Rice *et al.* (1995)** and **Ivanova *et al*. (2001)** and **Orf, Heba *et al*. (2006)** reported that , the production of the plant growth regulators like auxins, particularly indole-3-acetic acid (IAA) and indole-3-pyruvic acid, zeatin, zeatin riboside and reacted cytokinins by Methylotrophs and IAA production and nitrogen fixation by *Rhizobium* has been reported as the factors that enhances plant growth of legumes. The increase in the vegetative growth of the plant is attributed to the increase in the yield of a crop.

Table 10. Seed yield (kg plot^{-1}) of various chickpea genotypes as affected by application of different bacterial inoculation at harvest stage

Parameters / Genotypes	Seed yield (kg plot^{-1})								
	Un inoculated			R. inoculation			R.+ ppfm inoculation		
	S1	S2	x̄	S1	S2	x̄	S1	S2	x̄
GT1	0.63	0.73	0.68	1.16	1.20	1.18	1.25	1.37	1.31
GT2	1.40	1.61	1.51	1.66	1.85	1.76	1.72	1.90	1.81
GT3	1.70	1.85	1.78	1.95	2.10	2.03	1.97	2.01	1.99
GT4	1.72	1.20	1.46	1.75	1.84	1.89	1.85	1.90	1.87
GT5	1.26	1.30	1.28	1.64	1.96	1.80	1.75	1.97	1.86
GT6	1.22	1.19	1.21	1.56	1.65	1.61	1.65	1.72	1.69
GT7	1.51	1.35	2.43	1.98	1.83	1.89	2.10	2.15	2.13
G195	1.76	1.65	1.71	2.25	2.50	2.38	2.30	2.50	2.40
L.S.D	0.33	0.19	----	0.17	0.65	----	0.27	0.61	-----

S: season **GT:** Genotype

3.4. Seed yield (ton fed.$^{-1}$)

Data in Table (11) show that un-inoculated treatment recorded the lowest value of seed yield (0.252 ton fed.$^{-1}$) in both seasons as compared to other treatments. Inoculation with specific *Mesorhizobium* scored higher value (0.950 ton. fed.$^{-1}$) as compared to un-inoculated treatment in both seasons. Application with PPFMs as foliar spraying in the presence of rhizobial inoculation scored highest value (0.960 ton fed.$^{-1}$) as compared to rhizobial inoculation ones. Application of both rhizobial inoculations had a positive effect on seed yield (ton fed.$^{-1}$) for all tested chickpea genotypes as shown in table (11). Untreated treatments recorded the lowest seed yield values as compared to inoculated treatments as such or in-combination with PPFMs bacteria. The values were 0.810 ,0.718, 0.765 and 0.950 seed yield (ton fed^{-1}) for inoculated chickpea genotypes GT3, GT4, GT7 and G195 respectively and corresponding values at rhizobial inoculation in combination with PPFMs bacteria were 0.796 ,0.753, 0.850 and 0.960 (ton fed.$^{-1}$) in the same order. These data were in agreement with those obtained by **(Kantar *et al*., 2003; Ozturk *et al*., 2003)** and **Orf, Heba *et al*. (2006)** who reported that significant increases in seed protein content due to bacterial inoculation supported the hypothesis that biological nitrogen fixation by the Rhizobium and PGPR-root associations could be responsible for the observed higher N uptake of inoculated plants.

Senthilkumar *et al.* (2002) and Shehata, Sawsan (2006) established The increase in the yield due to compatible nature of *Methylobacterium* & *Rhizobium* and they found that, combined influence on phyllosphere by methylotrophs, which are plant growth promoting phyllosphere (PGPP) bacteria, and on rhizosphere by *Rhizobium*, which is nitrogen fixing bacterium, might have resulted in increased plant growth and yield parameters.

Table 11. Seed yield (ton fed^{-1}) of various chickpea genotypes as affected by application of different bacterial inoculation at harvest stage

Parameters / Genotypes	Seed yield (ton fed^{-1})								
	Un inoculated			R. inoculation			R. + ppfm inoculation		
	S1	S2	$\bar{x}$	S1	S2	$\bar{x}$	S1	S2	$\bar{x}$
GT1	0.252	0.292	0.772	0.464	0.504	0.484	0.500	0.548	0.524
GT2	0.560	0.644	0.602	0.664	0.740	0.702	0.686	0.760	0.724
GT3	0.680	0.740	0.710	0.780	0.840	0.810	0.788	0.804	0.796
GT4	0.688	0.480	0.584	0.700	0.736	0.718	0.740	0.760	0.753
GT5	0.504	0.520	0.512	0.656	0.784	0.720	0.700	0.788	0.744
GT6	0.488	0.476	0.482	0.624	0.660	0.642	0.660	0.688	0.674
GT7	0.604	0.540	0.572	0.780	0.732	0.756	0.840	0.860	0.850
G195	0.704	0.660	0.682	0.900	1.00	0.950	0.920	1.00	0.960
L.S.D	0.168	0.153	-----	0.128	0.191	-----	0.182	0.175	----

S: season **GT:** Genotype

CONCLUSION

In the present study it could be concluded that: under Egyptian soil conditions, necessity exists for inoculation with specific rhizobia alone or in combination with PGPR bacteria to maximizing the development and yield production of chickpea plants. All tested genotypes of chickpea emphasized the superiority of response to inoculation with specific rhizobia and foliar application with PPFM bacteria. PPFMs did support nodule form, plant growth and yield and reduce using chemical fertilizers specially nitrogen fertilizers. GT3, GT4 and GT7 chickpea genotypes gave a positive results and higher values for the all tested chickpea parameters in comparison to chickpea G195 variety under Egyptian soil conditions.

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