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Evaluation Impact of Some Plant Growth Promoting Microorganisms on the Growth and Productivity of Cowpea

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ABSTRACT

Two field experiments were conducted during two successive summer seasons of 2012 and 2013 at the experimental farm infected with root-knot nematodes (RKN) *Meloidogyne* sp. and *Fusarium* sp., at El-Kassasein Research Station, Ismallia Governorate, Egypt, to investigate the effect of microbial inoculation with *Pseudomonas fluorescens* , *Bacillus subtilis*, *Rhodotorula mucilaginosa, Candida tropicalis* and *Saccharomyces cerevisiae* in comparison with chemical nematicide on soil microbial counts, soil enzyme activities, number of *Meloidogyne* sp. larva in soil and roots and *Fusarium* indication, growth parameters, dry weight, yield and its components as well as chemical constituents of cowpea**.** Treating cowpea plants with chemical nematicides significantly decreased total bacterial count and soil enzymes activities and significantly increased vegetative growth, dry weight of leaves, branches and whole plant, seed yield and it's components, nitrogen and protein percentage. Microbial inoculation of cowpea plants with *Pseudomonas fluorescens+Bacillus subtilis+ Saccharomyces cerevisiae* significantly increased total bacterial count and soil enzymes activities (Dehydrogenase, Nitrogenase and Chitinase) while, the treatment with *P. fluorescens + B. subtilis + Candida tropicalis* caused most reduction in number of nematode larva in soil and roots and *Fusarium* indication in soil. Moreover, significant increases in vegetative growth traits (plant height, number of leaves and branches per plant as well as leaf area), dry weight of leaves, branches and whole plant, root system parameters, seed yield and it's components, nitrogen and protein percentage by microbial inoculation of cowpea plants with *P. fluorescens + B. subtilis + Candida tropicalis*.

Key words: Cowpea, microbial inoculation, soil enzymes, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Rhodotorula mucilaginosa, Candida tropicalis, Saccharomyces cerevisiae*, *Meloidogyne* sp., *Fusarium* sp., growth parameters and yield.

Introduction

Cowpea (*Vigna unguiculata* L.Walp) is widely cultivated by millions of people in the tropics as a major source of their livelihoods (Fang *et al.*, 2007). Although cowpea is cultivated worldwide, over 75% of the world production is obtained from Africa (Singh *et al.,* 2002). Cowpea is cultivated globally primarily as a vegetable, cover and cash crops, it is rich in quality protein and has energy content almost equivalent to that of cereal grains. The protein in cowpea seeds is rich in lysine and tryptophan compared to cereal seeds (Rabia *et al*., 2015). Cowpea grain is consumed directly after cooking, or as a component of meals made from cereals or root crops. It cakes (made from mashed and fried seed) are also sold as a fast food along roadsides in humid forest of South-western Nigeria (Gideon and Owoeye, 2013). Cowpea is attacked by many diseases caused by viruses, bacteria, fungi, and nematodes (Killani *et al.,* 2011 and Oliveira *et al.,* 2014). *Fusarium* genus species are among the most economically important phytopathogenic and mycotoxigenic fungi in the world. (Akhtyamova and Sattarova, 2013). Moreover, Root-Knot Nematodes (RKN) *Meloidogyne sp.* causes root damage by direct feeding and establishment, which in turn leads to plant wilting and chlorosis in approximately 3000 plant species (Ruiz *et al.,* 2014). Nematode population in the field has been suppressed and kept at levels below the economic threshold by nematicide application, planting resistant crop varieties, fallowing, intercropping, heat treatment, use of biological agents, soil amendments and all cultural practices that are inhibitory to nematode development and reproduction (Daramola *et al.,* 2015). Chemical nematicides are prohibited due to their potentially detrimental effects on the environment and human health even though they have been shown to be effective for inhibiting nematode infestation. Biological control of nematodes and pathogenic fungi is therefore an attractive option with the aim of maintaining current cultivation practices and minimizing damage to the environment. (Zhang *et al.,* 2008).

Plant growth promoting microorganisms increase plant growth indirectly either by suppression of well-known diseases caused by major pathogens or by reducing the deleterious effects of minor pathogens (micro-organisms, which reduce plant growth without obvious symptoms) (Morsy *et al.,* 2010). The following

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rhizospheric environment and microbial antagonistic activities can be highlighted: (1) synthesis of hydrolytic enzymes, such as chitinases, glucanases, proteases, and lipases, that can lyse pathogenic fungal cells (Neeraja *et al.,* 2010 and Maksimov *et al.,* 2011), (2) competition for nutrients and suitable colonization of niches at the root surface (Kamilova *et al.,* 2005), (3) regulation of plant ethylene levels through the ACC-deaminase enzyme, which can act to modulate the level of ethylene in a plant in response to stress imposed by the infection (Van Loon, 2007), and (4) production of siderophores and antibiotics (Katiyar and Goel, 2004). Bacteria and yeast are common inhabitants of soil and plant, by various mechanisms, they may affect the growth of pathogens and reduce disease incidence (Leite *et al.,* 2005). For example, *Bacillus* sp. and *Pseudomonas* sp. are pathogens of nematodes and plant pathogenic fungi and also promote plant growth (Li *et al.,* 2005; Killani *et al.,* 2011 and Ruiz *et al.,* 2014). On the other hand, yeasts exhibit plant growth promoting characteristics, including pathogen inhibition (El-Tarabily and Sivasithamparam, 2006); phytohormone production (Morsy *et al.,* 2014) and phosphate solubilization (Amprayn *et al.,* 2012); stimulation of mycorrhizal- root colonization (Alonso *et al.,* 2008) and may influence plant growth indirectly by encouraging the growth of other plant growth promoting rhizo-microorganisms, through vitamin B_{12} production (Medina *et al.,* 2004).

The aim of this study is evaluation of some plant growth promoting microorganisms such as (*Pseudomonas fluorescens*, *Bacillus subtilis, Saccharomyces cerevisiae, Candida tropicalis* and *Rhodotorula mucilaginosa*) as biofertilizer and biocontrol agents on growth plant and yield of cowpea growing in infected soil with root-knot nematodes (RKN) *Meloidogyne* sp. and *Fusarium* sp.

Materials and Methods

The present investigation was conducted at the Agriculture Research Farm, El-Kassasien Hort. Res. Station, Ismalia Governorate, Egypt, during two summer seasons of 2012 and 2013, to study the effect of microbial inoculation in comparison with chemical nematicides on soil microbial counts, soil enzyme activities, number of nematode larva in soil and roots and *Fusarium* indication, growth parameters, dry weight, yield and its components as well as chemical constituents of cow pea (*Vigna unguiculata* L. Walp.) cv. Kaha1.
The physical and chemical analysis of the experimental soil is presented in Table (1) according to Chapman and Pratt (1982).

Table 1: The physical and chemical properties of the experimental soil (average of two seasons).

Physical properties		Chemical properties	
Coarse sand (%)	5.9	Organic matter (%)	0.29
Fine sand (%)	78.8	Available K (ppm)	119.3
Silt (%)	8.6	Available P (ppm)	4.85
Clay (%)	6.7	Available N (ppm)	21.7
Field capacity (%)	8.5	Calcium carbonate (%)	3.97
Wilting point (%)	4	pH	7.8
Available water (%)	5.5	EC $dS.m^{-1}$(1:5)	0.59
Water holding capacity (%)	14.5	S.P.	23.5

This experiment included 11 treatments as follow (all bacteria and yeast treatments were added on its Media):
T1-*Pseudomonas fluorescens* T2- *Bacillus subtilis* T3-*Rhodotorula mucilaginosa*.
T4- *Candida tropicalis.* T5- *Saccharomyces cerevisiae*.
T6- *Pseudomonas fluorescens* + *Bacillus subtilis.*
T7- *Pseudomonas fluorescens* + *Bacillus subtilis* + *Rhodotorula mucilaginosa.*
T8- *Pseudomonas fluorescens* + *Bacillus subtilis* + *Candida tropicalis*.
T9- *Pseudomonas fluorescens* + *Bacillus subtilis* + *Saccharomyces cerevisiae.*
T10- Chemical nematocides
T11- Control (untreated).

These treatments were distributed in a randomized complete block design with three replications. Seeds of cow pea were obtained from Hort. Res. Inst., Agric. Res. Center, Egypt, and sown on March 16th and 19th in 2012 and 2013, respectively on one side of drippers lines (two seeds /hill) at 25 cm apart. At 15 days from sowing, plants were thinned leaving one plant / hill. The experimental unit area was 10.5m^2 with 60 plants, it contained 5 dripper lines with 3m length each with 70 cm wide. Three inner rows were possessed for yield determination, whereas the two outer rows were for determination of plant growth characters. One dripper line was left between each two experimental units without treating as a guard row. All experimental units received equal amounts of fertilizers at rates of 40 Kg N/fed. as ammonium sulphate (20.5% N), 30 Kg P/fed. as calcium super phosphate (15.5% P_2O_5) and 40 Kg K/fed. as potassium sulphate (48% K_2O), respectively. Other cultural practices control were carried out according to the recommendations of Ministry of Agriculture.
Data recorded: The obtained data in this study were recorded as follows:

Plant growth parameters:

Six plants from each plot were randomly taken at 50 days after sowing to evaluate the following vegetative growth characters: Plant height, number of branches/plant, number of leaves/plant. Leaf area (cm^2/ plant) was calculated according to Koller (1972) using following formula:

$$\text{Plant leaf area } cm^2 = \frac{\text{Dry weight of leaves} \times \text{Disk area of 10 disks } (cm^2)}{\text{Dry weight of 10 disks}}$$

Dry weight:

Cowpea parts (branches and leaves) were oven dried at 70 ºC till constant weight. The dry weight of leaves and branches/ plant as well as whole plant were determined.

Root system traits:

A random sample of three plants from every experimental unit was taken after 50 days from sowing and the root of cowpea plants were carefully separated by washing the sand from them and roots were placed in a flat glass dish containing a little amount of water. Roots were straighted with forceps, so that they can not overlap and were held in position, according to Helal and Sauerbesk (1986), and the following data were recorded per root: root length (cm), dry weight of root (g), number and fresh weight of nodules as well as root volume (cm^3).

Yield and its components

Dry pods of each plot were harvested at suitable maturity stage counted and weighed in each harvest till the end of the experiment and the following data were recorded:
Average number of pods per plant, average number of seeds per pod, weight of seeds / pod , seed yield/plot (kg) and total seed yield/fed (Kg).

Seed Mineral Contents (NPK)

Dried seeds at second harvest were finely ground separately and digested with sulfuric acid and percholoric acid (3:1). Nitrogen, phosphorus and potassium were determined according to the methods described by Bremner and Mulvaney (1982), Olsen and Sommers (1982) and Jackson (1970), respectively.

Total Crude Protein (%)

The previously determined nitrogen of dry seeds was used for calculating total crude protein percentage by multiplying N-values by 6.25 (AOAC, 1980).

Microorganisms:

Pseudomonas fluorescens and *Bacillus subtilis* were kindly provided by Microbiology Res. Departement, Soils, Water and Environment Research Institute, ARC. Giza, Egypt. Conical flasks (250 ml) containing 100 ml of King's broth medium (King *et al*., 1954) for *Pseudomonas fluorescens* and nutrient broth medium (Difco, 1985) for *Bacillus subtilis* were sterilized at 121°C for 15 min were used as growth medium. The flasks were inoculated with a loop- full of the tested strain then incubated at 28-30°C on rotary shaker (150 rpm) for 2 days. Bacterial inoculants (10^9 CFU/ml) were added at rate of 10 L/fed. three times at 20, 40 and 60 days. While chemical nematicides was done by using furadan at 20kg/fed.

Rhodotorula mucilaginosa, Candida tropicalis and *Saccharomyces cerevisiae*, were kindly provided by Microbiology Res. Departement, Soils, Water and Environment Research Institute, ARC. Giza, Egypt. The strains were grown individual on glucose peptone yeast extract agar (GPY) medium (Difco, 1985). The strains inoculated in 250 ml Erlenmeyer flasks containing 50 ml of liquid glucose peptone yeast extract (GPY) medium. Then, flasks were incubated at 30°C for 48h on a rotary shaker 150 rpm. Yeasts inoculants (10^9 CFU/ ml) were added at rate of 10 L/ fed three times at 20, 40 and 60 days of planting with water irrigation.
Data recorded: The obtained data in this study were recorded as follows:

Soil microbiological activity:

Samples of soil were taken from the rhizospheric zone of cowpea plants roots after 50 and 80 days from planting to recorded population dynamics of total bacterial, yeast count, total nitrogen fixer bacteria count, *Pseudomonas* sp count, chitinase, nitrogenase and dehydrogenase activity.

The total bacterial count , yeast count and *Pseudomonas* sp count were determined by the plate count method according to Reinhold *et al.* (1985) using nutrient ager medium for total bacterial count ; Glucose Peptone Yeast extract agar (GPY) medium for yeast count and King's agar medium for *Pseudomonas* sp. count (Difco, 1985). The total count of nitrogen fixers was determined by the most probable number (CFU/g soil)

method described by Cochran (1950) using Watanabe medium (Watanabe and Barraquio, 1979). Nitrogenase, dehydrogenase and chitinase activities in rhizosphere were also determined according to Dilowarth (1970), Skujins (1976) and Rodriguez-Kabana *et al.* (1983), respectively.

Identification of soil born disease:

Samples of soil were taken from the rhizospheric zone of cow pea plants roots after 50 and 80 days from planting to record the reduction on number of juveniles in soil and roots according to Norton (1978) and *Fusarium* indication was determined by the plate count method according to Reinhold *et al.* (1985) using Potato Dextrose Agar (PDA) medium (Difco, 1985).

Statistical analysis

The obtained data were statistically analyzed by using MSTAT statistical software and the treatments means were compared by using LSD at 0.5 level of probability according to Snedecor and Cochran (1980).

Results

Soil microbial counts

Regarding to Table 2. the microbial count was increased after microbial inoculations. The total bacterial count was highly increased after inoculation with *Pseudomonas fluorescens* + *Bacillus subtilis* + *Saccharomyces cerevisiae* which recorded 159 and 182 CFU×10^7/ g dry soil after 50 and 80 days of planting, respectively in the first season. Also the second season was showed the same trended which recorded 168 and 198 CFU×10^7/ g dry soil after 50 and 80 days of planting respectively. The obtained results also revealed an increase of *Pseudomonas* sp. count and the increase was recorded in treatment T9 which recorded 150 and 163 CFU×10^5/ g dry soil after 50 and 80 days of planting, respectively in the first season. The same trend was showed in the second season which recorded 158 and 175 CFU×10^5/ g dry soil after 50 and 80 days of planting respectively. Moreover, the total count of yeast was in the treatment inoculated with yeast specially *Saccharomyces cerevisiae* with recorded which recorded 33 and 43 CFU×10^4/ g dry soil after 50 and 80 days of planting, respectively in the first season. The same trend was showed in the second season which recorded 35 and 44 CFU×10^4/ g dry soil after 50 and 80 days of planting respectively. Moreover, the total nitrogen fixer count was increased after microbial inoculation the most increased was observed in treatment inoculated with *Pseudomonas fluorescens* + *Bacillus subtilis* + *Saccharomyces cerevisiae* which recorded 11 and 17 CFU×10^4/ g dry soil after 50 and 80 days of planting respectively at first season and 13 and 18 CFU×10^4/ g dry soil after 50 and 80 day of planting respectively at the second season.

Table 2: Effect of microbial inoculation on some microbial counts in the rhizophere of cowpea plants during 2012 and 2013 seasons.

Characters / Treatments	Total bacterial count (CFU×10^7/ g dry soil)				*Pseudomonas sp.* count (CFU×10^5/ g dry soil)				Total yeast (CFU×10^4/ g dry soil)				Total nitrogen fixer counts (CFU X10^4/ g dry soil)			
	Season															
	1st		2nd		1st		2nd		1st		2nd		1st		2nd	
	Days															
	50	80	50	80	50	80	50	80	50	80	50	80	50	80	50	80
Pseudomonas fluorescens (P.f)	89	104	92	123	67	78	73	85	1	3	2	5	3.2	3.9	3.4	4
Bacillus subtilis (B.s)	68	95	72	108	35	54	39	67	1	9	3	11	4.2	5.3	4.5	5.4
Rhodotorula mucilaginosa (R. m)	119	132	123	148	68	73	78	124	15	19	17	26	5.3	6.4	5.6	7.2
Candida tropicalis (C.t)	130	156	143	176	90	124	106	134	19	24	23	31	7.2	9.5	7.9	9.2
Saccharomyces cerevisiae (S.c)	140	167	152	183	80	103	87	127	33	43	35	44	9.5	14	13	17
P.f + B.s	76	92	85	108	90	114	93	136	5	8	9	11	4	5.4	4.2	6.3
P.f + B.s + R.m	144	161	156	176	96	136	102	153	18	26	23	37	6.3	7.8	6.4	8.2
P.f + B.s + C.t	128	173	143	185	131	148	142	163	24	33	27	42	7.9	9.5	8.1	9.5
P.f + B.s + S.c	159	182	168	198	150	163	158	175	159	182	168	198	11	17	13	18
Chemical nematicide	35	56	42	51	23	47	38	56	2	6	3	9	0.42	0.54	0.41	0.64
Control (untreated)	59	69	63	78	52	65	57	78	10	14	12	19	1.2	1.3	1.3	1.7

CFU = Colony - Forming Units

Soil enzyme activities

Soil enzyme activities (Dehydrogenase, Nitrogenase and Chitinase) were significantly increased after microbial inoculation as we see in Table 3. For example Dehydrogenase recorded the highest increased in treatment with *Pseudomonas fluorescens* + *Bacillus subtilis* + *Saccharomyces cerevisiae* which recorded 93.8 and 132.1 µg TPF / g dry soil / day after 50 and 80 days of planting at the first season. While, the second season who the same trend it was recorded 101.9 and 147.7µg TPF / g dry soil / day after 50 and 80 days of planting. Moreover, Nitrogenase was significantly increased in all treatments and the most increased was recorded after inoculation with *Pseudomonas fluorescens* + *Bacillus subtilis* + *Saccharomyces cerevisiae* which recorded 45.5 and 54.2 µ mole C_2H_4/g soil/h after 50 and 80 days of planting. Also, the second season has the same trend it

was recorded 48.5 and 59.3 µ mole C_2H_4/g soil/h after 50 and 80 days of planting. On the other hand Chitinase activity was significant increased espials in yeast treatments the most increased was recorded in treatment with *Pseudomonas fluorescens + Bacillus subtilis + Saccharomyces cerevisiae* which recorded 3.42 and 4.07 µg NAGA/g dry soil/h after 50 and 80 days of planting at the first season. But, the second season was recorded 3.93 and 4.5 µg NAGA/g dry soil/h after 50 and 80 days of planting.

Table 3: Effect of microbial inoculation on some soil enzyme activities of cowpea plants during 2012 and 2013 seasons.

Characters / Treatments	Dehydrogenase activity (µg TPF/g dry soil/ day)				Nitrogenase activity (µ mole C_2H_4/g dry soil/h)				Chitinase activity (µg NAGA/g dry soil/h).			
	Season											
	1st		2nd		1st		2nd		1st		2nd	
	Days											
	50	80	50	80	50	80	50	80	50	80	50	80
Pseudomonas fluorescens (P.f)	37.6	62.2	40.7	74.3	36.6	41.7	37.3	43.5	2.90	3.37	3.13	3.63
Bacillus subtilis (B.s)	39.2	53.0	43.1	68.4	37.1	43.0	38.1	41.8	2.84	3.68	3.23	3.84
Rhodotorula mucilaginosa (R. m)	58.1	75.5	62.9	84.1	41.4	49.1	43.1	50.7	3.81	4.25	4.14	4.84
Candida tropicalis (C.t)	67.1	82.4	71.2	90.1	42.6	50.3	41.8	51.5	3.27	3.81	3.73	4.56
Saccharomyces cerevisiae (S.c)	77.9	89.4	82.1	127.8	46.5	52.4	46.4	53.4	3.03	3.61	3.41	4.15
P.f + B.s	43.6	78.8	53.9	63.7	37.7	42.3	38.2	46.3	2.81	3.82	3.13	4.30
P.f + B.s + R.m	76.1	108.1	72.3	121.6	40.3	48.3	31.6	52.2	3.12	3.93	3.71	4.50
P.f + B.s + C.t	83.4	123.9	92.5	137.0	41.1	50.2	41.9	51.2	3.14	3.82	3.25	3.71
P.f + B.s + S.c	93.8	132.1	101.9	147.7	45.5	54.2	48.5	59.3	3.42	4.07	3.93	4.50
Chemical nematicide	23.4	25.9	28.7	31.6	25.5	29.2	25.3	28.0	1.27	1.82	1.46	2.04
Control (untreated)	28.3	33.0	36.6	47.3	31.2	33.1	30.3	35.1	2.23	2.67	2.47	2.81
L.S.D at 0.05 level	1.95	3.66	2.3	2.54	1.9	1.8	1.4	2.1	0.18	0.13	0.13	0.18

Reduction of nematode larva in soil and roots and *Fusarium* indication

Regarding to data in Table 4. both of chemical pesticides and biological treatments caused reduction on nematode larva in soil and roots. The most biological treatments caused high reduction was *Pseudomonas fluorescens + Bacillus subtilis + Candida tropicalis* it was recorded 65% and 89% in soil and 67% and 95% in roots after 50 and 80 days of planting in the first season. While, the second season who the same trend it was recorded 63% and 92 % soil and 65% and 96% in roots after 50 and 80 day of planting. On the other hand *Fusarium* was found in the soil inoculated with yeasts only but the treatment with *Pseudomonas fluorescens + Bacillus subtilis* , mix of bacteria and mix of bacteria and yeast not indicated *Fusarium* after 50 days of planting. But after 80 days of planting *Fusarium* was not indicated in all microbial treatments.

Table 4: Effect of microbial inoculation on reduction of nematode larva in soil and roots and *Fusarium* indication of cowpea plants during 2012 and 2013 seasons.

Characters / Treatments	Reduction of nematode larva in soil (%)				Reduction of nematode larva in roots (%)				*Fusarium* indication			
	Season											
	1st		2nd		1st		2nd		1st		2nd	
	Days											
	50	80	50	80	50	80	50	80	50	80	50	80
Pseudomonas fluorescens (P.f)	50	75	52	78	62	82	65	84	-	-	-	-
Bacillus subtilis (B.s)	42	67	45	72	49	71	52	76	-	-	-	-
Rhodotorula mucilaginosa (R. m)	24	43	25	46	39	59	42	63	+	-	+	-
Candida tropicalis (C.t)	35	52	34	56	42	65	46	67	+	-	+	-
Saccharomyces cerevisiae (S.c)	25	49	23	51	40	62	43	63	+	-	+	-
P.f + B.s	48	72	47	73	51	73	49	69	-	-	-	-
P.f + B.s + R.m	54	78	58	73	57	85	60	83	-	-	-	-
P.f + B.s + C.t	65	89	63	92	67	95	65	96	-	-	-	-
P.f + B.s + S.c	60	85	59	87	65	92	68	93	-	-	-	-
Chemical nematicide	90	75	89	73	68	90	67	87	+	+	+	+
Control (untreated)	-	-	-	-	-	-	-	-	+	+	+	+

(+) was indicated, (-) was not indicated

Morphological characters

Data presented in Table 5. show clearly the effect of microbial inoculation in comparison with chemical nematicides on cowpea plant height, number of leaves and branches per plant as well as leaf area during both seasons of study. Such data indicated that bio-inoculation cowpea plants with the tested strains induced stimulative effect on vegetative growth parameters compared to control treatment. In this respect, treating cowpea plants with chemical nematicides significantly increased all the studied vegetative growth traits followed by inoculation plants with *Pseudomonas fluorescens + Bacillus subtilis + Candida tropicalis* without significant differences between them as compared to other treatments especially control treatment.

Table 5: Effect of microbial inoculation on morphological characters of cowpea plants during 2012 and 2013 seasons at 50 days of planting.

Characters / Treatments	Morphological characters / plant							
	Plant height (cm)		No. of leaves		No. of branches		Leaf area (cm^2)	
	Season							
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Pseudomonas fluorescens (P.f)	26.7	29.0	12.3	10.3	3.33	3.33	831.4	902.1
Bacillus subtilis (B.s)	30.3	36.0	15.0	11.7	4.00	3.67	1284.5	1353.7
Rhodotorula mucilaginosa (R. m)	29.7	32.3	13.0	16.3	4.33	4.67	1732.8	1761.4
Candida tropicalis (C.t)	30.3	36.0	13.0	11.7	3.33	3.33	1112.8	986.5
Saccharomyces cerevisiae (S.c)	27.7	33.3	13. 7	13.3	3.67	4.00	1494.3	1638.7
P.f + B.s	27.7	33.0	13. 3	16.0	3.33	4.00	1335.4	1511.5
P.f + B.s + R.m	29.7	35.7	14.3	14.7	3.67	4.00	1764.6	1665.0
P.f + B.s + C.t	27.7	36.7	15.3	17.0	4.00	4.33	1844.1	2113.7
P.f + B.s + S.c	26.7	34.3	13.7	13.0	3.67	4.00	1287.7	1320.5
Chemical nematicide	29.3	35.3	15.3	17.7	4.33	4.67	2193.7	2343.5
Control (untreated)	26.3	29.0	10.0	9.7	3.00	3.00	825.1	814.6
L.S.D at 0.05 level	2.9	2.5	2.5	2.6	1.24	1.27	152.8	190.3

Dry weight

Results in Table 6. illustrate the effect of microbial inoculation on cowpea dry weight expressed as dry weight of leaves, branches and whole plant during both seasons of 2012 and 2013 such data revealed that significant differences due to the tested treatments in both seasons compared to control treatment. In this connection, treating cowpea plants with chemical nematicides significantly increased dry weight of leaves, branches and whole plant followed by inoculation cowpea plants with *Pseudomonas fluorescens* + *Bacillus subtilis* + *Candida tropicalis* On the other side the lowest values were recorded with control treatment.

Table 6: Effect of microbial inoculation on dry weight of cowpea plants during 2012 and 2013 seasons at 50 days of planting.

Characters / Treatments	Dry weight / plant (g)					
	Leaves		Shoots		Total	
			Season			
	1st	2nd	1st	2nd	1st	2nd
Pseudomonas fluorescens (P.f)	8.23	7.67	2.51	2.84	10.74	10.51
Bacillus subtilis (B.s)	8.08	8.59	2.14	2.49	10.22	11.08
Rhodotorula mucilaginosa (R. m)	10.93	11.73	3.05	3.51	13.98	15.24
Candida tropicalis (C.t)	8.01	9.21	3.30	3.57	11.31	12.78
Saccharomyces cerevisiae (S.c)	9.47	10.39	2.97	2.86	12.44	13.25
P.f + B.s	8.40	9.55	2.83	2.66	11.23	12.21
P.f + B.s + R.m	11.19	11.13	4.13	4.21	15.32	15.34
P.f + B.s + C.t	11.62	13.66	4.27	5.51	15.89	19.17
P.f + B.s + S.c	10.13	11.33	4.01	4.19	14.14	15.52
Chemical nematicide	13.85	14.73	5.09	7.10	18.94	21.83
Control (untreated)	7.19	8.12	2.23	2.30	9.42	10.42
L.S.D at 0.05 level	1.62	1.23	0.67	0.69	1.96	1.51

Root system

Results in Table 7. illustrate the effect of bacterial inoculation in comparison with chemical nematicides on root system of cowpea plants expressed as root length, dry weight of root, number and fresh weight of nodules as well as root volume during both seasons of 2012 and 2013. It is obvious from the data that there were significant differences due to the tested treatments in both seasons of study on all root system parameters compared to chemical nematicides or control treatments. In this connection, treating cowpea plants with *Pseudomonas fluorescens* + *Bacillus subtilis* + *Candida tropicalis* significantly increased abovementioned characters of root system followed by inoculation with *Pseudomonas fluorescens* + *Bacillus subtilis* + *Saccharomyces cerevisiae* without significant differences between them in most cases. On the other side the lowest values in this respect were recorded by control treatment, while chemical nematicides gave the lowest values of number and fresh weight of nodules.

Seed yield and it's components

Data presented in Table 8 show clearly the effect of microbial inoculation on total seeds yield and it's components expressed as number of pods per plant, number and weight of seeds per pod, weight of hundred seeds and seed yield per plot as well as total seed yield per feddan. Concerning number of pods per plant, it is obvious from the data that treating cowpea plants with chemical nematicides significantly increased number of pods per plant which gave 25.7 and 27.3 in the 1st and 2nd seasons, respectively, followed by inoculation cowpea plants with *Pseudomonas fluorescens* + *Bacillus subtilis* + *Saccharomyces cerevisiae* which gave 25.3 and 27.3 in the 1st and 2nd seasons, respectively, and inoculation with *Pseudomonas fluorescens* + *Bacillus subtilis* + *Candida tropicalis* which recorded 24.1 and 27.1 in the 1st and 2nd seasons, respectively, without significant differences among them. Concerning of number seeds per pod, the results show that inoculation cowpea plants

with *Pseudomonas fluorescens* + *Bacillus subtilis* + *Saccharomyces cerevisiae* significantly affected number of seeds per pod which gave 10.0 in both seasons of study followed by *Pseudomonas fluorescens* + *Bacillus subtilis* + *Candida tropicalis* which gave 9.6 and 10.6 in the 1st and 2nd seasons, respectively, and chemical nematicides which recorded 9.8 and 10.0 in the 1st and 2nd seasons, respectively, without significant differences among them.

Regarding weight of seeds per pod it is obvious from the same data that treating cowpea plants with chemical nematicides significantly affected weight of seeds per pod and recorded 1.61 and 1.62g in the 1st and 2nd seasons, respectively, followed by inoculation cowpea plants with *Pseudomonas fluorescens* + *Bacillus subtilis* + *Candida tropicalis* which gave 1.69 and 1.62g and inoculation with *Pseudomonas fluorescens* + *Bacillus subtilis* + *Saccharomyces cerevisiae* which recorded 1.62 and 1.55g in the 1st and 2nd seasons, respectively, without significant differences among them. With respect to weight of hundred seeds the same results in Table 8 show that inoculation cowpea plants with different tested types of bacteria and yeasts had stimulative effect on weight of hundred seeds compared to control treatment. In this rearguard, the highest values were recorded by inoculated cowpea plants with *Candida tropicalis* in both seasons of the study followed by inoculation with *Saccharomyces cerevisiae* in the first season and treatment with chemical nematicide in the second season. With regard to seed yield per plot and total seed yield per feddan it is clear from the same data that treating cowpea plants with chemical nematicides significantly increased seed yield per plot and total seed yield per feddan which gave 2.482 and 2.556 kg for seed yield per plot and 992.8 and 1022.4 kg for total seed yield per fed. followed by inoculation with *Pseudomonas fluorescens* + *Bacillus subtilis* + *Candida tropicalis* which recorded 2.446 and 2.523 kg for seed yield per plot and 978.5 and 1009.2 kg for total seed yield per feddan and inoculation with *Pseudomonas fluorescens* + *Bacillus subtilis* + *Saccharomyces cerevisiae* which gave2.304 and 2.490 kg for seed yield per plot and 921.9 as well as 996.0 kg for total seed yield per feddan in the 1st and 2nd seasons, respectively, without significant differences among the three treatments as compared to control.

Generally, it was observed that number of pods per plant, number and weight of seeds per pod, seed yield per plot and total seed yield per feddan of cowpea were gradually increased by treating plants with chemical nematicides or inoculation with *Pseudomonas fluorescens* + *Bacillus subtilis* + *Candida tropicalis* or inoculation with *Pseudomonas fluorescens* + *Bacillus subtilis* + *Saccharomyces cerevisiae* without significant differences among them in most cases as compared to other treatments especially control treatment which recorded the lowest values in this respect.

Table 7: Effect of microbial inoculation on root system characters of cowpea plants during 2012 and 2013 seasons at 50 days of planting.

Characters / Treatments	Root system / plant									
	Root length (cm)		Root dry weight (g)		Root volume (cm^3)		Number of nodules		Nodules fresh weight (g)	
	Season									
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Pseudomonas fluorescens (P.f)	12.2	12.8	1.54	1.63	10.75	12.26	12.67	13.11	0.588	0.610
Bacillus subtilis (B.s)	12.8	13.5	1.63	1.72	11.38	13.17	13.45	13.78	0.639	0.637
Rhodotorula mucilaginosa (R. m)	13.3	14.4	1.69	1.83	11.84	13.86	14.11	15.48	0.658	0.725
Candida tropicalis (C.t)	13.7	14.9	1.74	1.90	12.27	14.63	15.81	16.40	0.738	0.761
Saccharomyces cerevisiae (S.c)	14.1	14.5	1.80	1.85	12.65	14.04	14.34	15.91	0.667	0.739
P.f + B.s	13.6	15.0	1.72	1.91	12.10	14.51	14.82	16.18	0.689	0.748
P.f + B.s + R.m	14.0	15.7	1.78	2.00	12.48	15.19	14.91	16.54	0.691	0.768
P.f + B.s + C.t	16.3	18.0	2.06	2.29	12.46	17.45	17.82	17.93	0.832	0.836
P.f + B.s + S.c	14.7	17.4	1.86	2.21	13.25	16.78	16.29	17.11	0.756	0.782
Chemical nematicide	13.0	14.3	1.64	1.82	11.56	13.69	6.12	6.25	0.281	0.299
Control (untreated)	11.7	12.3	1.46	1.56	10.15	11.65	9.67	10.21	0.445	0.473
L.S.D at 0.05 level	0.9	1.0	0.26	0.33	0.67	0.43	0.66	0.51	0.014	0.17

Table 8: Effect of microbial inoculation on yield and it's components of cowpea plants during 2012 and 2013 seasons.

Characters / Treatments	Yield and it's components											
	No. of pods / plant		No. of seeds / pod		Weight of seeds / pod (g)		Weight of 100 seeds (g)		Yield / plot (kg)		Yield / feddan (kg)	
	Season											
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Pseudomonas fluorescens (P.f)	19.7	21.1	8.6	9.3	1.49	1.37	16.35	14.45	1.763	1.736	705.3	694.3
Bacillus subtilis (B.s)	20.1	21.6	8.4	9.1	1.51	1.40	16.34	15.33	1.823	1.819	729.5	727.7
Rhodotorula mucilaginosa (R. m)	21.3	23.0	8.2	9.5	1.50	1.25	17.81	14.62	1.920	1.729	768.0	691.7
Candida tropicalis (C.t)	20.7	22.3	7.6	8.7	1.58	1.48	18.29	17.02	1.965	1.985	786.2	793.9
Saccharomyces cerevisiae (S.c)	23.7	25.1	9.2	10.0	1.57	1.51	18.20	15.10	2.235	2.276	894.1	910.5
P.f + B.s	24.3	25.9	9.8	10.0	1.53	1.38	18.10	14.75	2.229	2.148	891.7	859.1
P.f + B.s + R.m	23.0	24.7	9.6	8.6	1.54	1.25	16.86	15.07	2.123	1.856	849.3	742.6
P.f + B.s + C.t	24.1	27.1	9.6	10.6	1.69	1. 62	16.83	15.52	2.446	2.523	978.5	1009.2
P.f + B.s + S.c	25.3	27.3	10.0	10.0	1.62	1.55	16.67	14.64	2.304	2.490	921.9	996.0
Chemical nematicide	25.7	27.3	9.8	10.0	1.61	1.62	16.70	15.59	2.482	2.656	992.8	1022.4
Control (untreated)	18.5	19.1	8.4	8.6	1.33	1.36	15.09	14.21	1.477	1.560	591.0	623.9
L.S.D at 0.05 level	2.8	3.6	1.0	0.9	0.08	0.13	0.99	0.85	0.315	0.407	127.1	156.0

Chemical constituents of seeds

Data presented in Table 9 show clearly the effect of microbial inoculation on chemical constituents of cowpea seeds expressed as nitrogen, phosphorus and potassium as well as protein percentage during both seasons of 2012 and 2013, such data indicated that bio-inoculation of cowpea plants with different tested types of microorganisms had stimulative effect on seeds chemical constituents compared to control treatment. In this regard, inoculation cowpea plants with *Pseudomonas fluorescens* + *Bacillus subtilis*. gave the highest values of nitrogen and protein percentage followed by inoculation with *Pseudomonas fluorescens* + *Bacillus subtilis* + *Candida tropicalis* .

Regarding seed phosphorus and potassium content the same data (Table 9) showed also that, treating cowpea plants with chemical nematicide or inoculation with different types of bacteria and yeasts did not reflect significant effect on phosphorus and potassium percentage in both seasons of study.

Table 9: Effect of microbial inoculation on chemical constituents of cowpea seeds during 2012 and 2013.

Characters / Treatments	Chemical constituents (%)							
	N		P		K		Protein	
	Season							
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Pseudomonas fluorescens (P.f)	3.37	3.49	0.425	0.465	2.43	2.54	21.1	21.8
Bacillus subtilis (B.s)	3.32	3.46	0.458	0.445	2.59	2.65	20.8	21.6
Rhodotorula mucilaginosa (R. m)	3.48	3.78	0.450	0.460	2.49	2.65	24.3	24.9
Candida tropicalis (C.t)	3.75	3.91	0.428	0.460	2.54	3.03	23.4	24.4
Saccharomyces cerevisiae (S.c)	3.71	3.82	0.403	0.435	2.81	3.03	23.2	23.9
P.f + B.s	3.85	3.86	0.431	0.470	2.70	2.86	24.1	24.1
P.f + B.s + R.m	3.64	3.70	0.406	0.440	2.70	2.76	22.8	23.1
P.f + B.s + C.t	3.84	3.89	0.454	0.475	2.49	2.65	24.0	24.3
P.f + B.s + S.c	3.76	3.78	0.421	0.445	2.54	2.65	23.5	23.6
Chemical nematicide	3.56	3.62	0.482	0.495	2.64	2.81	22.3	22.6
Control (untreated)	3.34	3.45	0.421	0.445	2.34	2.54	20.9	21.5
L.S.D at 0.05 level	0.29	0.47	0.083	0.090	N.S	N.S	1.76	2.18

Correlation study

Data presented in Table 10. show the simple correlation coefficient between total seed yield and its components of cowpea plants. The results obtained indicated that total seeds yield (kg/feddan) showed positive and highly significant correlation with number of pods per plant (0.960^{**} and 0.918^{**}), number of seeds per pod (0.749^{**} and 0.813^{**}), weight of seeds per pod ($0.815^{**}$and 0.829^{**}) and seed yield per plot (1.000^{**} and 1.000^{**}) in the 1st and 2nd season, respectively. These results are in a good line with those reported by Arisha *et al.* (2015). Number of pods per plant recorded positive and highly significant correlation with number of seeds per pod and yield per plot (kg) but it showed only positive significant correlation with weight of seeds per pod (g) and gave 0.666^{*} and 0.551^{*} in 1st and 2nd seasons, respectively.

Moreover, number of seeds per pod exhibited positive and highly significant correlation with yield per plot (kg), but it did not reflect any significant correlation effect with weight of seeds per pod (g) in the 1st season, while it exhibited positive significant (0.582^{*}) with weight of seeds per pod in the 2nd season.

Again weight of seeds per pod (g) recorded positive and highly significant correlation with yield per plot (kg) and gave 0.845^{**} and 0.829^{**} in 1st and 2nd seasons respectively.

Table 10: Some correlation coefficient between yield and its components of cowpea plants during 2012 and 2013 seasons.

		Season 2012				Season2013			
		1	2	3	4	1	2	3	4
Y	Total yield (kg/fed.)	0.960^{**}	0.749^{**}	0.895^{**}	1.000^{**}	0.918^{**}	0.813^{**}	0.829^{**}	1.000^{**}
1	No. of pods /plant		0.849^{**}	0.666^{*}	0.960^{**}		0.777^{**}	0.551^{*}	0.918^{**}
2	No. of seeds /pod			$0.370^{N.S}$	0.749^{**}			0.582^{*}	0.813^{**}
3	Weight of seeds /pod (g)				0.845^{**}				0.829^{**}
4	Yield /plot (kg)								

NS= Not significant $_{*}$ *= Significant* $_{**}$*= Highly significant*

Economic returns:

Data presented in Table 11. Showed that economic performance of cowpea plants as affected by bacterial inoculation in comparison with chemical nematicides.The results showed that the highest net return (3780.1£E fed^{-1}) was obtained under the combined addition of *Pseudomonas fluorescens* + *Bacillus subtilis* + *Candida tropicalis* such treatment returns the highest benefit-cost ratio (1.73) in comparison with other treatments, thus this treatment proved to be the economical for cowpea production under the conditions of this study.

Table 11: Economic performance of cowpea plants as affected by bacterial inoculation in comparison with chemical nematicides during 2012 and 2013 seasons.

Treatments	yield (Kg fed^{-1})(1)	Gross return (£E fed^{-1})(2)	Treatment cost (£E fed^{-1})(3)	Total variable cost (£E fed^{-1})(4)	Net return (£E fed^{-1})(5)	Benefit cost ratio(6)	Order
Pseudomonas fluorescens (P.f)	699.8	6298.2	300	5215	1083.2	1.21	10
Bacillus subtilis (B.s)	728.6	6557.4	300	5215	1342.4	1.26	9
Rhodotorula mucilaginosa (R.m)	729.9	6569.1	150	5065	1504.1	1.30	8
Candida tropicalis (C.t)	790.1	7110.9	150	5065	2045.9	1.40	6
Saccharomyces cerevisiae (S.c)	902.3	8120.7	150	5065	3055.7	1.60	4
P.f + B.s	875.4	7878.6	300	5215	2663.6	1.51	5
P.f + B.s + R.m	796.0	7164.0	250	5165	1999.0	1.39	7
P.f + B.s + C.t	993.9	8945.1	250	5165	3780.1	1.73	1
P.f + B.s + S.c	959.0	8631.0	250	5165	3466.0	1.67	2
Chemical nematicides	1007.6	9068.4	600	5515	3553.4	1.64	3
Control (untreated)	607. 5	5476.5	-	4915	561.5	1.11	11

(1) Cowpea yield as average of two seasons, (2) Gross return as marketable yield (kg/ fed^{-1}) x 9000 £E /Ton, (3) Treatment cost was calculated according to the following prices: Rhodotorula mucilaginosa, Candida tropicalis. and Saccharomyces cerevisiae = 150 £E /fed. each one of them, Pseudomonas fluorescens 300 £E / fed. and Bacillus subtilis 300 £E / fed., (4) Total variable cost (£E/fed^{-1}) including: Treatment cost plus land leasehold, transplants, N and P fertilizers, microelements, pesticides, labors and other agricultural practices, which equal nearly 4915 £E// fed^{-1}. (5) = (2)-(4). (6) = (2)/ (4).

Discussion

In this study, five tested microorganisms, (*Pseudomonas fluorescens*, *Bacillus subtilis, Saccharomyces cerevisiae, Candida tropicalis* and *Rhodotorula mucilaginosa*) were investigated for their role as a PGPR and effectiveness for controlling *Meloidogyne* sp. and *Fusarium* sp. under filed conditions. As shown in the results the growth of cowpea (*Vigna unguiculata* L.Walp) planted in soil infected with *Meloidogyne* sp. and *Fusarium* sp. when treated with PGPR gave higher growth and yield in comparison with control treatments. The increase of total microbial count, *Pseudomonas fluorescens* count and total yeast count in the rhizosphere of cowpea plants proved that inoculation with *Pseudomonas fluorescens*, *Bacillus subtilis, Saccharomyces cerevisiae, Candida tropicalis* and *Rhodotorula mucilaginosa* increased the soil microbial population (Botha, 2011). Concerning the activity of dehydrogenase activity, data cleared a close correlation between activity of dehydrogenase activity and microbial population (Tolba *et al.,* 2010). Moreover, nitrogenase and chitinase activities were significant increased, it may attributed to yeasts in the root zone may influence other plant growth promoting rhizo-microorganisms, through vitamin B_{12} production (Medina *et al.,* 2004). Also, soil inoculation with *Pseudomonas* sp. significantly increased soil enzyme activities (Sharma *et al.,* 2011).

Bacteria and yeasts are important as antagonists of soil pathogens such as *Meloidogyne* sp. and *Fusarium* sp. The reduction in No. of nematode larva in soil and roots could be attributed to the suppression of *Pseudomonas fluorescens* against *Meloidogyne* sp. that inhibited egg hatching and caused mortality of the juveniles by producing wide variety of antibiotics, siderophores, hydrogen cyanide (HCN) and protease, which caused inhibition of egg hatching and killing juveniles (Zehnder *et al.,* 2001 and Siddiqui *et al.,* 2005). On the other hand, *Bacillus* has great diverse nature including antibiotic production, nitrogen fixation, degradation of protein and good plant growth promoting activities along with biological control of various fungal diseases involving various mechanisms such as antibiosis and lysis. Hence on the basis of functions of various microorganism soil may be classified as disease inducing, disease-suppressive, zymogenic and synthetic soils (Pankaj *et al.,* 2012). *Bacillus* control a wide range of plant pathogens including *Fusarium* species by secretion of a number of metabolites including antibiotics, volatile compound HCN, siderophores, enzymes chitinase and B-1, 3-glucanase (Killani *et al.,* 2011). *Bacillus subtilis* showed the high antagonistic activity on *M. incognita*. These results were agreements with Ruiz *et al.* (2014) who suggest that the cell-free culture filtrate of *Bacillus subtilis* might be able to contain toxic metabolites against J2 *M. incognita* nematode. The presence of toxic components in cell-free culture filtrates of *Bacillus* spp. on nematode immobility and subsequent mortality has been previously reported (Caneiro *et al.,* 1998). Also, this bacterium produces hydrolytic enzymes such as glucanases or proteases and the antibiotic lipopeptides surfactin, fengycin, and/or iturin A, capable of acting against fungi and nematodes (Killani *et al.,* 2011).

Recently many species of *Rhodotorula* have significant inhibitory effect against plant pathogens such as *Fusarium* genus, and growth stimulating effect on some agricultural crops. (Akhtyamova and Sattarova, 2013). Moreover, the mode of action of *Candida* sp. as a biocontrol agent against root knot nematodes, could be attributed to production of antipathogens diffusible metabolites and cell-wall degrading enzymes (EL-Tarabily and Sivasithamparan, 2006). In addition, it has the ability to produce toxins (El-Mehalawy, 2004). These findings were agreements with Morsy *et al.* (2010) who reported that *Pseudomonas fluorescens* and *Candida* sp. had effectiveness for controlling root- knot nematode (RKN) *Meloidogyne* sp., in addition their role as a PGPR in tomato planted in soil infested with *Meloidogyne* sp. Moreover, the enhancement of plant emergence by *Pseudomonas fluorescens* and *Rhodotorula mucilaginosa* may be attributed to the secretion of some substances

on the plants that may activate the biological process and accelerate the emergence. Promoting of plant growth by the bioagents could be resulted from facilitating uptake of nutrients by roots. It was reported that PGPR promote plant growth directly through nitrogen fixation, phosphorus solubilization and production of phytohormones like auxin ,cytokinin, ethylene, indole-3- acetic acid and gibberellic acid, and indirectly by suppressing soil borne pathogens (Rakib *et al.,* 2013) .

Furthermore, contents of NPK, plant growth and yield were also higher in plants treated with tested microbs. It could be attributed to, soil yeasts representing the genera *Candida*, *Saccharomyces* and *Rhodotorula* have a great role as bio-fertilizers (Botha, 2011). Increases in the level of inorganic nutrition after decomposition of *Saccharomyces cerevisiae* inoculant enhanced biologically derived CO_2 production were proposed to explain partly the multiple effect of yeast culture (Nikolay *et al.,* 2001). In addition, these yeasts may be able to solubilize insoluble phosphates thus making these nutrients more readily available to plants (Botha, 2011). Furthermore, contents of N, P and K were also higher in plants inoculated with *Saccharomyces cerevisiae* in soil. The increasing of N, P and K levels affected positively the plant growth, in addition to the increase of total yeast count in the cowpea rhizosphere. This could be explained on the basis that yeasts are capable of indirectly enhancing the plant growth (El-Tarabily and Sivasithamparam, 2006 and Cloete *et al.,* 2009). Also, *S. cerevisiae* can produce the auxin indole-3-acetic acid (IAA) and gibberellins (Morsy *et al.,* 2014). And *Candida* sp. could producing indole-3-acetic acid (IAA), gibberellins and possibility of other PGPR. (El-Tarbily, 2004). The auxin indole-3-acetic acid is best known for its role in plant cell elongation, division, and differentiation (Reeta *et al.,* 2010). Plant performance can also be increased as a result of the production of plant growth regulators compounds includes indole-3-acetic acid, indole-3-pyruvic acid, gibberellins and polyamines by yeasts (Botha, 2011). Moreover, Singh *et al*. (1991) found that inoculation of legumes with *Saccharomyces cerevisiae* increases nodulation as well as *Arbuscular mycorrhiza* (AM) fungal colonization therefore a variety of yeasts are known to occur in the rhizosphere (Botha, 2006 and Botha 2011), and the interaction between mycorrhizal fungi and soil yeasts is expected. Thus, the mutualistic symbioses between mycorrhizae and plant roots may facilitate uptake of up to 80% of the phosphorus and 25% of the nitrogen requirements of the host plant (Marschner and Dell, 1994). On the other hand, *Rhodotorula* has significant growth stimulating effect on some agricultural crops. (Akhtyamova and Sattarova, 2013 and Rakib *et al.,* 2013).

Moreover, contents of N, P, K, plant growth parameters and yield were also higher in plants treated with bacteria. It could be attributed to, *Pseudomonas fluorescens* has a vital role as a PGPR, by producing biologically active substances or the conversion of unavailable minerals and organic compounds into forms available to plants besides, the plant growth promotion ability of *Pseudomonas* is a function of good colonization of roots (Rodriguez and Fraga, 1999), production of growth hormones (Siddiqui and Futai, 2009), associative N_2 fixation (Hong *et al.*, 1991); solublizing nutrients such as P (Whitelaw, 2000), promoting mycorrhizal function (Garbaye, 1994), regulating ethylene production in rhizosphere (Glick, 1995); releasing phytohormones (Beyeler *et al.,* 1999).

These results are agreement with Gholami *et al.* (2009) found that *Pseudomonas fluorescens* significantly increased Plant height, seed weight, number of seed per ear and leaf area, shoot dry weight in Maize (*Zea mays* L.). Also, Ahemad and Khan (2012) who found that *Pseudomonas* sp. significantly increased plant dry weight, nodules numbers, total chlorophyll content, leghaemoglobin, root N, shoot N, root P, shoot P, seed yield and seed protein of Greengram (*Vigna radiata* (L.) wilczek). Moreover, Sharma *et al.* (2011) reported that *Pseudomonas* sp. significantly increased total productivity, and nutrient uptake in Soybean and wheat. On the other hand, *Bacillus* sp. enhance the plant growth and yield by direct and indirect via Plant Growth Promoting (PGP) activities and nitrogen fixation (Killani *et al.,* 2011). These findings were agreement with Beneduzi *et al.* (2012) who reported that *Bacillus* sp. significantly promoted the root and shoot growth in rice plants. Also, Wani and Khan (2010) reported that *Bacillus* species significantly improved growth, nodulation, chlorophyll, leghaemoglobin, seed yield and grain protein in Chickpea (*Cicer arietinum*) plants.

On the other hand, chemical nematicide caused increased in growth parameters and yield and reduced the number of nematodes in soil and roots. This is in agreement with the findings of Tanimola (2008) who reported that the best plant growth, higher yield and nematode control on cowpea plots treated with carbofuran at 2 kg /ha, as compared with untreated nematode-infected cowpea.

Bio-fertilizers are low cost, effective and renewable source of plant nutrients to supplement chemical fertilizers (Agamy *et al*., 2013). In addition to their role in enhancing the growth of the plants, biofertilizers can act as biocontrol agents in the rhizosphere at the same time. This synergistic effect, when present, increases the role of application of bio-fertilizers in the sustainable agriculture (Agamy *et al*., 2013).

Conclusion

From the previous results of this investigation, it could be concluded that microbial inoculation of cowpea plants with *Pseudomonas fluorescens* + *Bacillus subtilis* + *Candida tropicalis* significantly enhanced total bacterial count , soil enzymes activities, plant growth, dry weight and yield and its components, nitrogen and protein percentage and caused reduction on number of *Meloidogyne* sp larva in soil and roots and *Fusarium*

indication in soil instead of chemical nematicides to reducing environmental pollution and contamination the underground water**.**

References

Agamy R., M. Hashem and S. Alamr, 2013. Effect of soil amendment with yeasts as bio-fertilizers on the growth and productivity of sugar beet. African Journal of Agricultural Research, 8(1) : 46-56.

Ahmed, M. and M. S. Kahan, 2012. Alleviation of fungicide-inducal phytotoxicity in greengram (*Vigna radiata* (L.) wilczek) using fungicide- tolerant and plant growth promoting *Pseudomonas* strain. Saudi J. Biol. Sci., 19: 451-459.

Akhtyamova, N. and R. Sattarova, 2013. Endophytic yeast *Rhodotorula rubra* strain TG-1: Antagonistic protection activities. Biochemistry & Physiology, 2:1-5.

Alonso, L. M., D. Kleiner and E. Ortega, 2008. Spores of the mycorrhizal fungus Glomus mosseae host yeasts that solubilize phosphate and accumulate polyphosphates. Mycorrhiza, 18: 197-204.

Amprayn, K., M. T. Rose, M. Kecskés, L. Pereg, H. T. Nguyen and I. R. Kennedy, 2012. Plant growth promoting characteristics of soil yeast (*Candida tropicalis* HY) and its effectiveness for promoting rice growth. Appl. Soil Ecol., 61: 295-299.

A.O.A.C., 1980. Association of Official Agriculture Chemists Official Method of Analysis 13th Ed. Washington, D.C.

Arisha, H. M. E., K. A. M. Nour and F. M. A. El-Saadony, 2015. Comparative study on antioxidants and synthetic plant growth regulators effects for reducing flower shedding, enhancing yield in relation to leaf anatomical structure of pepper plant proceeding. The 2nd International Conference on Horticulture crops, 15-18 March, Cairo, Egypt.

Beneduzi, A., A. Ambrosini and L. M. P. Passaglia, 2012. Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. Genetics and Molecular Biology, 35, (4), 1044-1051.

Beyeler, M., C. Keel, P. Michaux and D. Haas, 1999. Enhanced production of indole-3-acetic acid by a genetically modified strain of *Pseudomonas fluorescens* CHA0 affects root growth of cucumber, but does not improve protection of the plant against *Pythium* root rot. FEMS Microbiology Ecology, 28: 225-233.

Botha, A., 2006. Yeast in soil. In: Rosa CA, Péter G (Eds.). The Yeast Handbook; Biodiversity and Ecophysiology of Yeasts. Springer-Verlag, Berlin., pp. 221-240.

Botha, A., 2011. The importance and ecology of yeasts in soil. Soil Biol. Biochem., 43: 1-8.

Bremner, J. M. and C. M. Mulvaney, 1982. Total nitrogen. In [Page, A.L., R.H. Miller, and D.R. Keeney] (Eds). Methods of Soil Analysis, Part 2 Amer. Soc. Agron. Madison. WI-W.S.A. p. 595-624.

Caneiro, R. M., I. Souza and L .C. Belarmino, 1998. Nematicidal Activity of *Bacillus* spp. Strains on Juveniles of *Meloidogyne javanica Nematologia.* Brasileira, 22: 12- 21.

Chapman, H .D. and F. Pratt, 1982. Methods of Soil Analysis". Part 2 A.S.S. Madison wiscoasin.

Cloete, K. J., A. J. Valentine, M. A. Stander, L. M. Blomerus and A. Botha, 2009. Evidence of symbiosis between the soil yeast *Cryptococcus laurentii* and a sclerophyllous medicinal shrub, *Agathosma betulina* (Berg.) Pillans. Microbial Ecol., 57: 624-632.

Cochran, W.G., 1950. Estimation of bacterial densities by means of the most probable number. Biometrics, 6: 105-116.

Daramola, F., S. Afolami, O. Enikuomehin, C. Omonhinmin and A. Adebayo, 2015. Nematicidal effects of carbofuran and GC-MS analysis of its residue in pineapple fruits. Int. J. Agric. Biol., 17: 357-362.

Dilowarth, M. J., 1970. The acetylene reduction method for measuring biological nitrogen fixation. Rhizobium News Letter, 15 (7): 155-163.

Difco, M., 1985. Dehydrated culture media and reagents for microbiology, pp. 621. In Difco Loaboratories (Ed.). Incorporated Detroit. Michigan, 48232 USA.

El-Mehalawy, A. A., 2004. The rhizosphere yeast fungi as biocontrol agents for wilt disease of kidney bean caused by *Fusarium oxysporum.* International J. Agric. & Boil., 6(2): 310-316.

El-Tarabily, K. A., 2004. Suppression of *Rhizoctonia solani* diseases of sugar beet by antagonistic and plant growth-promoting yeasts. J. Appl. Microbiol. 96: 69–75.

El-Tarabily, K. A., K. Sivasithamparam, 2006. Potential of yeasts as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. Mycosci. 47: 25–35.

Fang, J., C. C. Chao, P. A. Roberts and J. Elhers, 2007.Genetic diversity of cowpea [*Vigna unguiculata* (L.)Walp.] in four West African and USA breeding programs as determined by AFLP analysis. Genetic Resources and Crop Evolution, 54(6): 1197-1209

Garbaye, J., 1994. Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytologist, 128: 197-210.

Glick, B. R., 1995. The enhancement of plant growth by free-living bacteria. Can. J. Microbiol., 41: 109-117.

Gideon, I. O. and A. E. Owoeye, 2013. In vitro control of Anthracnose disease of cowpea (*Vigna unguiculata* L. Walp) caused by *Colletotrichum destructivum* O'Gara with *Cyathula prostrata* L. and *Diodia scandens* SW leaf extracts. J. Intercult Ethnopharmacol, 2 (1): 29-36.

Gholami, A., S. Shahsavani and S. Nezarat, 2009. The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. Int. J. Biol. Life Sci., 1, 35–40.

Helal, H. M. and D. Sauerbesk, 1986. Entwicklung und Aktivitat des Wurzelsysteme in Abhangigheit von der Bodendichte . Landw Forsch., 20: 381- 388.

Hong, Y., J. J. Pasternak and B. R. Glick, 1991. Biological consequences of plasmid transformation of the plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2. Can. J. Microbiol., 37: 796-799.

Jackson, M. L., 1970. Soil chemical analysis. Prentice Hall, Englewood Cliffs, N.J.

Kamilova F., S. Validov, T. Azarova, I. Mulders and B. Lugtenberg, 2005. Enrichment for enhanced competitive plant root tip colonizers selects for a new class of biocontrol bacteria. Environ Microbiol 7: 1809- 1817

Katiyar, V. and R. Goel, 2004. Siderophore-mediated plant growth promotion at low temperature by mutant of fluorescent pseudomonad. Plant Growth Regul. 42: 239- 244.

Killani, A. S., R. C. Abaidoo, A. K. Akintokun; and M. A. Abiala, 2011. Antagonistic Effect of Indigenous *Bacillus subtilis* on Root-/Soil-borne Fungal Pathogens of Cowpea. Researcher, 3(3): 11- 18.

King, E.O., M. K. Ward and D.E. Raney, 1954. Two simple media for the demonstration of pyocyanin and flourescein. J. Lab. Clin. Med., 44: 301- 307.

Koller, H. R., 1972. Leaf area – leaf weight relationship in the soybean canopy. Crop. Sci., 12 (3/4) : 180-183.

Leite, L.G., S. B. Alves, A. Batista and D. W. Roberts, 2005. Simple inexpensive media for mass production of three entomophthoralean fungi. Mycological Research, 109: 326-334.

Li, B., G. L. Xie, A. Soad and J. Coosemans, 2005. Suppression of Meloidogyne javanica by antagonistic and plant growth promoting rhizobacteria. J Zhejiang Univ Sci 6(B): 496–501.

Maksimov, I.V., Abizgil'dina, R.R. and L. I. Pusenkova, 2011. Plant growth promoting rhizobacteria as alternative to chemical crop protectors from pathogens (Review). Appl. Biochem. Microbiol. 47: 333-345.

Marschner, H. and B. Dell, 1994. Nutrient uptake in mycorrhizal symbiosis. Plant Soil, 59: 89-102.

Medina, A., M. Vassileva, F. Caravaca, A. Roldan and R. Azcon, 2004. Improvement of soil characteristics and growth of *Dorycnium pentaphyllum*by amendment with agrowastes and inoculation with AM fungi and/or the yeast *Yarowia lipolytica*. Chemosphere. 56: 449–456.

Morsy Ebtsam M.; El Batanony Nadia H. and O. N. Massoud, 2014. Improvement of soybean growth and productivity by inoculation with two yeast species in new reclaimed sandy soil amended with humic acid. African J. Microbiol. Res., 8 (46): 3794- 3803.

Morsy Ebtsam M., Neveen M. Galal and Hager I. Tolba, 2010. Biological control of root-knot nematode by some microorganisms on tomato plants. N. Egypt. J. Microbiol., 26: 130-145.

Neeraja, C., K. Anil, P. Purushotham; K. Suma, P. Sarma, B.M. Moerschbacher and A.R. Podile, 2010. Biotechnological approaches to develop bacterial chitinases as a bioshield against fungal diseases of plants. Crit. Rev. Biotechnol., 30:231-241.

Nikolay, V.; V. Maria; R. Azcon and M. Almudena, 2001. Application of free and Ca-alginate entrapped *Glomus deseticola* and *Yarowia lipolytica* in a soil-plant system. J. Biotecnology, 91: 237- 242.

Norton, D.C., 1978. Ecology of plant parasitic nematodes. John Willey and Sons, New York, USA. 238 pp.

Oliveira, J. T. A., H. Jose, T. B. Araujo-Filho , D. M. F. Grangeiro , J. Gondim and P. Segalin, 2014. Enhanced Synthesis of Antioxidant Enzymes, Defense Proteins and Leghemoglobin in Rhizobium-Free Cowpea Roots after Challenging with *Meloydogine incognita.* Proteomes, *2*: 527-549

Olsen, S.R. and L.E. Sommers, 1982. Phosphorus In [Page, A.L., R.H. Miller, and D.R. Keeney (Eds.). Methos of Soil Anaylsis, Part 2- Amer. Soc. Agron. Madison. WI- USA pp. 403-430].

Pankaj, K., K. Satyajeet, and R.C. Dubey, 2012. Diversity of Bacilli from Disease Suppressive Soil and their Role in Plant Growth Promotion and Yield Enhancement. New York Science J., 5 (1): 90- 111.

Rabia, B., B. Bisma, A. Anum, M. Saman, A. Amina and P. Sadia, 2015. Amelioration of salt affected soils for cowpea growth by application of organic amendments. J. of pharmacognosy and phytochemistry, 3(6): 87-90.

Rakib A. A., M. A. Athab and O. N. Matny, 2013. Management of potato virus Y (PVY) in potato by some biocontrol agents under field conditions. Advances in Environmental Biol., 7 (3): 441- 444.

Reeta, P. R., H. Ally, K. Olga and N. Jennifer, 2010. Aberrant Synthesis of Indole-3-Acetic Acid in *Saccharomyces cerevisiae* Triggers Morphogenic Transition, a Virulence Trait of Pathogenic Fungi. Genetics, 185: 211–220.

Reinhold, B., T. Hurek and L. Fendrik, 1985. Strain-specific chemotaxis of *Azospirillum* spp. J. Bacteriol. 162:190-195.

Rodriguez-Kabana, R., G. Godoy, G. Morgan-Jones and R.A. Shelby, 1983. The determination of soil chitinase activity: Conditions for assay and ecological studies. Plant and Soil, 75: 95-106.

Rodriguez, H. and R. Fraga, 1999.Phosphate solublizing bacteria and their role in plant growth promotion. Biotechnol. Adv., 17: 319- 339.

Ruiz, S. E.; A. J. Cristóbal; R. A. Reyes; S. J. Tun; R. A. García and A. J. Pacheco, 2014. In vitro antagonistic activity of *Bacillus subtilis* strains isolated from soils of the Yucatan Peninsula against *Macrophomina phaseolina* and *Meloidogyne incognita.* FYTON, 83: 45-47.

Sharma, S. K., B. N. Johri, A. Ramesh, O. P. Joshi and S.V. S. Prasad, 2011. Selection of plant growth-promoting *Pseudomonas* spp. that enhanced productivity of soybean-wheat cropping system in central. India. J. Microbiol. Biotechnol., 21: 1127–1142.

Siddiqui, Z.A. and K. Futai, 2009. Biocontrol of *Meloidogyne incognita* on tomato using antagonistic fungi, plant-growth-promoting rhizobacteria and cattle manure. Society of Chemical Industry Pest Manag Sci., 65: 943-948.

Singh, B. B., J. D. Elhers, B. Sharma and F. R. Freire Filho, 2002. Recent progress in cowpea breeding. Page 22-40. In: Challenges and opportunities for enhancing sustainable cowpea production. Proceedings of the world cowpea conference 111 held at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria; 4-8 September 2000:433pp.

Singh, C. S., A. Kapoor and S. S. Wange, 1991. The enhancement of root colonization of legumes by vesicular-arbuscular mycorrhizal (VAM) fungi through the inoculation of legume seed with commercial yeast (*Saccharomyces cerevisiae*). Plant Soil, 131: 129-133.

Skujins, J., 1976. Extracellular enzymes in soil. CRC Crit. Rev. Microbiol., 4: 383-421.

Snedecor, G. W. and W. G. Cochran (1980). Statistical Methods. 7th Ed. The Iowa State Univ., Press, Amer., Iowa , USA .

Tanimola, A.A., 2008. Comparison of the Effects of Carbofuran and Poultry Manure in the Management o*f Meloidogyne incognita* Race 2 on the growth and yield of Root-knot infected Cowpea (*Vigna unguiculata* (L. Walps). *J. Agric. Soc. Res.,* 8 (1):15-17.

Tolba, Hager I., Morsy, Ebtsam M. and El-Saida. H. El-badawy, 2010. Impact of microbial inoculation on maize (*Zea mays*) productivity under different levels of potassium fertilization. Egypt. J. Biotechnol., 35: 172-184.

Van Loon L.C., 2007. Plant responses to plant growth-promoting rhizobacteria. Eur. J. Plant Pathol., 119:243-254.

Watanabe, I. and W. Barraquio, 1979. Low levels of fixed nitrogen required for isolation of free N_2-fixing organisms from rice roots. Matura (London), 277: 565- 566.

Wani, P. A. and M. S. Khan, 2010. Bacillus species enhance growth parameters of chickpea (*Cicer arietinum* L.) in chromium stressed soils. Food Chem. Toxicol., 48: 3262–3267.

Whitelaw, M. A., 2000. Growth promotion of plants inoculated with phosphate-solublizing fungi. Advances In Agronomy, 69: 99-151.

Zehnder, G. W.,J. F. Murphy, E. J. Ikora and J. W. Kloepper, 2001. Application of rhizobacteria for induced resistance. Eur. J. plant pathol., 107: 39-50.

Zhang, L., J. Zhang, P. Christie and X. Li, 2008. Pre-inoculation with Arbuscular mycorrhizal fungi suppresses root knot nematode (*Meloidogyne incognita*) on cucumber (*Cucumis sativus*). Biol. Fertil. Soils, 45: 205–211.