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Plant growth promoting rhizobacteria (PGPR) as eco-friendly alternatives for management root-knot nematodes, *Meloidogyne* spp. on tomato plants.

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Abstract

Plant Growth Promoting rhizobacteria (PGPR) are a various group of bacteria that could be create in the rhizosphere, at root surfaces and in association with roots. PGPR can be used as bio-control agents. The objective of this study was to assess four different bacterial strains i.e. *Pseudomonas fluorescens*, *Azospirillum brasilense*, *Azotobacter chroococcum* and *Bacillus megaterium* against *Meloidogyne* spp at three different incubation times i.e. 24, 48, 72 and 168 hrs under laboratory conditions. Furthermore, the strains were evaluated at different application times i.e. (one week before, at the same time and one week after nematode inoculation) as bio- control agents against *Meloidogyne* spp. on tomato plants in pots experiments. *In vitro* results revealed that the highest inhibition percentage in egg hatching and larvae mortality recorded after 168 hrs with *B. megaterium* by 17.3% and 92%, respectively. Greenhouse, results cleared that all evaluated bacterial strains at all different application times had the potentiality in *Meloidogyne* spp. parameters reduction i.e. (number of galls, egg masses, females/root system and juveniles in soil) and the reproduction to a huge extent compared to treated plants with nematode alone. Plants inoculated with *B. megaterium* achieved also the highest increases in shoot and

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root lengths and weights when applied one week before nematode inoculation by 213.3, 175, 180 & 291.3%, respectively compared to the other application times. Also it the highest values of ch.a, chl.b, total chlorophyll and carotenoids by 1.0, 0.75, 1.75 and 0.78 mg/ g.f.w., respectively recorded with *B.megaterium* when applied one week before nematode inoculation after 30 days from transplanting. Plants inoculated with *A. chroococcum* recorded the lowest photosynthetic pigments. Peroxidase (POX) and polyphenol oxidase (PPO) were increased with all bacterial strains treatments. The significant increase of dehydrogenase activity and total count of bacteria of all different bacterial strains observed when applied at all three application times were compared to plants infected with nematode alone.

Keywords: *Meloidogyne* spp., PGPR, Tomato (*Solanum lycopersicum*) , Photosynthetic pigments, Antioxidant enzymes.

INTRODUCTION

Root-knot nematodes (RKN) are the most essential nematode pests worldwide due to their great damage resulted on the very wide host range which contains more than 2000 plant species **(Gugino *et al*., 2008).** Root-knot nematodes *Meloidogyne* spp. are common in Egypt and worldwide and cause severe crop harmful especially in light soils **(Abd-Elgawad and Aboul-Eid , (2001).** The achievement of nematode reproduction on their appropriate hosts depends on the successful formation of such feeding sites which rely on the availability of appropriate concentrations of some chemicals and enzymes **(Baldacci-Cresp *et al*., 2012)** to be available in host tissues. PGPR is a diverse group of free-living soil bacteria that colonize rhizosphere and contribute to plant growth promotion which in turn increase the yield of agriculture

crops **Siddiqui and Shaukat (2002)**. PGPR plays significant role in the growth of sustainable agriculture system and is one of the main components in integrated nematode management **(Teymouri *et al.*, 2016)**. Egg-parasitic fungi, nematode trapping fungi, bacteria, and polyphagous predatory nematodes are biological control agents that have been evaluated **(Kerry & Hidalgo-Diaz, 2004; Kiewnick and Sikora, 2005; Abdelmoneim, 2006**). PGPR have exposed positive effects on tomato fruit quality, mainly on size and texture **(Hortencia *et al*., 2007).** Plant growth promoting substances from PGPR strains directly stimulus their host plant growth by inducing the cell division, tissue improvement, physiological and biochemical metabolisms. Though, these PGPR strains provide other maintenance to the host plant to cope up with nematode infection **(Mhatre *et al*., 2019)**. The present study was approved out to evaluate the effect four bacterial strains i.e. *P. fluorescens*, *A. brasilense*, *A. chroococcum* and *B. megaterium* on tomato plants performance infected with root knot nematodes *Meloidogyne* spp. under greenhouse conditions at three different application times (one week before, at the same time and one week after nematode infection).

MATERIALS AND METHODS

Microbial Inoculants

Bacterial strains included *P. fluorescens*, *A. brasilense*, *A. chroococcum* and *B. megaterium* were obtained from the Department of Agricultural Microbiological Research, Soils, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt. For inoculum preparation, a loop of each bacterium kindly was transferred into nutrient broth (NB) according to **Difco Manual (1985)** and was incubated for 24hr at 28^0C, and cell pellets were collected by centrifuging at 6000 rpm for 10 minutes after the cells were washed in sterile distilled

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water and re-suspended in 400 ml phosphate buffer pH 7. After 24 h, the turbidity of each bacterial culture was measured at wave length 600 nm and adjusted to 0.5 OD (10^7cfu /ml).

Preparation of *Meloidogyne* spp. inoculum

Root-knot nematodes *Meloidogyne* spp. was multiplied on tomato plants (*Lycopersicon esculentum* Mill. Cv. GS) which was transplanted in plastic pots with diameter 30 cm filled with non-sterilized sandy soil under greenhouse conditions at the experimental farm, Faculty of Agric., Menoufia Univ., Shebin El-Kom. Four months after, the infested roots by *Meloidogyne* spp., were used to prepare nematode inoculum as described by **Hussey and Barker (1973).**

Egg masses of *Meloidogyne* spp. were stained by the protocol described by **(Daykin and Hussey, 1985)**, while gall index measuring was determined according to the scale (0-10) of **(Bridge and Page, 1981)**.

***In vitro* experiment :**

Egg hatching and Larvae mortality of *Meloidogyne* spp .

The hatched eggs percentage was enumerated as follows:

Hatched eggs percentage = Number of hatched eggs in each treatment/ Total number of eggs x100.

The mortality percentage of larvae was enumerated as follows:

Larvae mortality percentage = Number of dead larvae in each treatment / Total number of larvae x 100

Greenhouse Experiments:

Tomato seeds (*Lycopersicon esculentum Mill* cv. GS) were obtained from a commercial nursery to perform the experiment. The seedlings were transplanted in black bags of polyethylene at the rate of (one seedling /pot) filled with 2 kg sandy-loamy soil mixture at the rate of (2:1; v/v). The experiment was designed as a complete randomized blocks with

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three replicates. Plants were daily watered and fertilized weekly with 5 ml of 2g /L of N:P:K (20:20:20) as recommended dose for Egyptian Ministry of Agriculture's. Eight weeks after nematode inoculation, plants were uprooted and their roots gently washed under tap water. The nematode inoculum was added at the same time of transplanting at the rate of 3000 eggs/plant in all pots except 3 pots served as a control. The eggs were inoculated in three holes around the root zone by pipetting. Fourteen treatments were carried out by applying each bacterium of all four bacterial strains at three different times with 6 pots served as a positive and negative control.

Plant biometrics was estimated at 30 and 60 days from transplanting, this included measurement of growth characteristics i.e. shoot and root length (cm) , root and shoot fresh weights (g) as well as shoot dry weight (g).

Photosynthetic pigments determination

Total chlorophyll amounts, chlorophyll a, chlorophyll b and carotenoids pigments were determined in the leaves of plants by the method of **Arnon (1949)**.

Antioxidant Enzymes

Peroxidase (POX) and polyphenol oxidase (PPO) enzymes activity was determined according to **(Chance and Maehly, 1955**) and **Brooche (1954)** after 30 days from transplanting.

Dehydrogenase activity and total viable aerobic bacteria in rhizosphere soil:

Dehydrogenase activity in the soil was carried out according to **Thalman (1967)** while the total viable aerobic bacteria were determined according to the method described by **Vincent (1970).**

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Statistical Analyses

Least significant difference test was used to compare means using the statistical analysis software; CoStat (CoHort Software, U.S.A) version 6.4, as described by **Duncan (1955)**.

RESULTS

In vitro Experiments

Impact of four different bacterial strains on egg hatching of *Meloidogyne* spp.

The inhibition percentage of egg hatching of *Meloidogyne* spp. studied under laboratory conditions at 28^0C by using four liquid bacterial strains i.e. *P. fluorescens*, *A. brasilense*, *A chroococcum* and *B. megaterium* at different incubation times i.e. 24, 48, 72 and 168 hrs. Results revealed that all treatments exhibited nematicidal effects against egg hatching compared to control. *B. megaterium* recorded the highest inhibition percentage at all different incubation times , followed by *P.fluorescens* , *A. brasilense* and *A.chroococcum* , respectively compared to control. The highest inhibition percentage of egg hatching achieved after 168 hrs in all treatments. The lowest values recorded after 168 hrs by 17.3, 34, 42 and 50%, respectively compared to control as recorded 87.3% after 168 hrs as shown in Figure (1).

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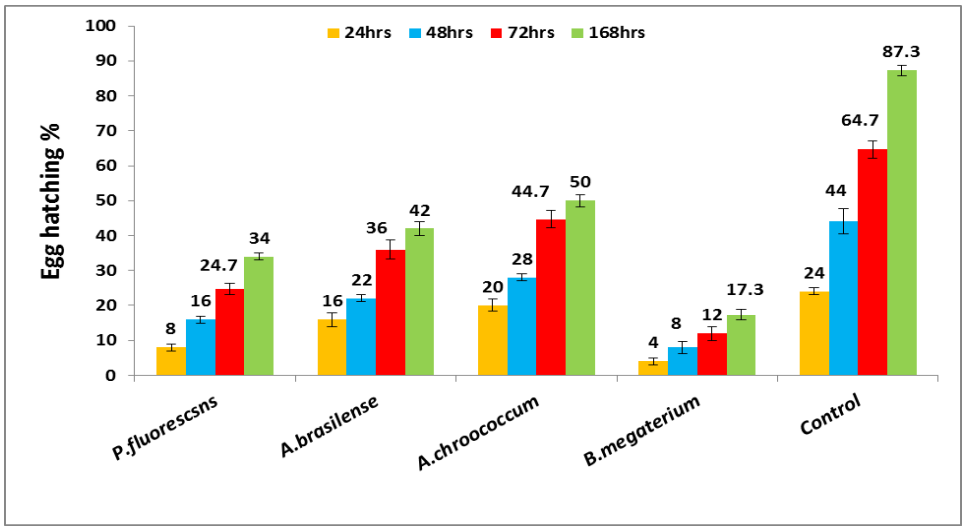

Figure (1): Egg hatching as affected by four bacterial strains at different times under laboratory conditions. Error bars represent the standard deviation between 3 replicates.

Impact of four different bacterial strains on juveniles mortality of *Meloidogyne* spp.

The juveniles mortality percentage of *Meloidogyne* spp. studied under laboratory conditions at 28^0C by using the liquid of four bacterial strains i.e. *P. fluorescens*, *A. brasilense*, *A. chroococcum* and *B. megaterium* at different incubation times i.e. 24, 48, 72 & 168 hrs. Results revealed that all treatments exhibited nematicidal effects on juveniles mortality compared to control. *B. megaterium* gave the highest percentage of juveniles mortality at all intervals times, followed by *P.fluorescens ; A.brasilense and A.chroococcum*. The highest mortality percentage value recorded after 168 hrs in all treatments. The highest mortality percentage of juveniles recorded 92% with *B. megaterium* after168 hrs , followed by *P.fluorescens* as recorded 80% and *A. brasilense* by 74% , while *A. chroococcum* recorded 64% compared to control by 16% as shown in Figure (2) .

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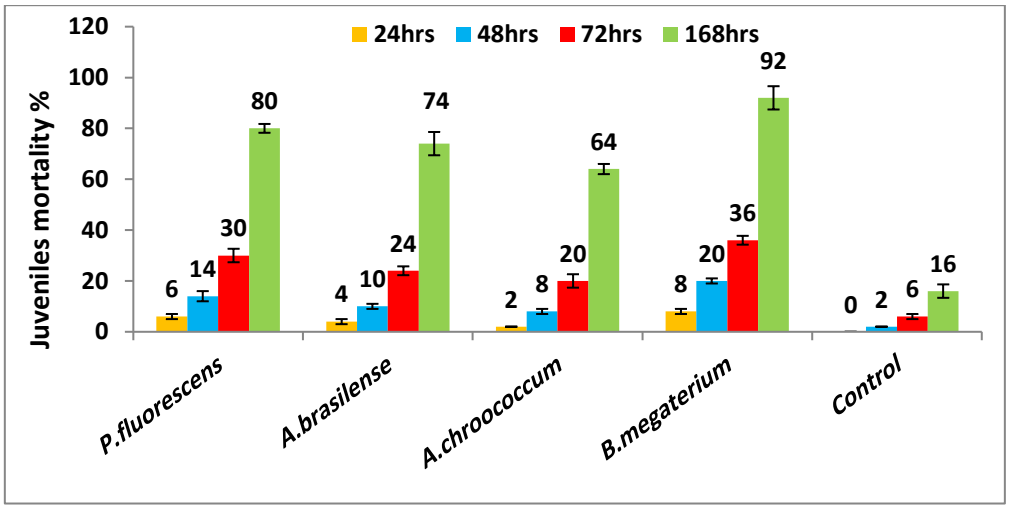

Figure (2): Juveniles mortality as affected by four bacterial strains at different incubation times under laboratory conditions. Error bars represent the standard deviation between 3 replicates .

In vivo experiments

Effect of four different bacterial strains at three applications times on management of *Meloidogyne* spp.

To evaluate the effect of four bacterial strains viz (*P. fluorescens, A. brasilense, A. chroococcum & B. megaterium*) against root-knot nematodes *Meloidogyne* spp.**,** a pot experiment was carried out under greenhouse conditions at three different application times (one week before, at the same time &one week after nematode inoculation) as presented in Table (1). Generally, results cleared that all bacterial strains had the potentiality decrease in *Meloidogyne* spp. parameters i.e. (galls, egg masses, females/root system and J_2 in soil) and the reproduction as well as the final population to a huge extent compared to treated plants with nematode alone. The reduction percentage varied among treatments according to the species of bacterial strains and application times also. Application of four bacterial liquid strains at one week before nematode inoculation gave the highest reduction in nematode parameters in soil and

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roots. The reduction percentages in nematode parameters in soil and roots were ranged between 62 to 93.3 %, respectively. The highest reduction in numbers of galls, egg masses, females/root system and J_2 / 250 g soil recorded with plants inoculated with *B. megaterium* at one week before nematode inoculation by 86, 88, 88.9 &93.3%, respectively, followed by the same bacteria at the same time of nematode inoculation by 85, 90, 86.1 & 92.5% , respectively , while when the bacteria was inoculated one week after nematode inoculation the reduction was 84.2 ,86 ,83.9 & 92% , respectively compared to plants treated with nematode alone. Inoculation plants with *P. fluorescens* decreased the number of galls, egg masses and females / root system and number of J_2 / 250 g soil by 83.3,84, 82.2 & 91.6%, respectively when it applied one week before nematode inoculation, followed by it applying at the same time of nematode inoculation by 82.6, 82, 80 & 91.2%, respectively compared to treated plants with nematode alone. Applying *P. fluorescens* one week after nematode inoculation recorded reduction percentages in galls, egg masses, and females / root system and J_2 / 250 g soil by 82, 79, 77.8 & 90.6%, respectively compared to plants treated with nematode alone. Similarly, applying *A. brasilense* at one week before of nematode inoculation achieved high results in decreasing the number of galls, egg masses, females in root system and J_2 in soil at the rate of 81.6, 77, 75.6 & 90.2% respectively, followed by applying this bacterium at the same time of nematode inoculation by 80, 75, 73.3 & 89.7% , respectively, then applying the bacterium one week after nematode inoculation by 79, 72, 71.7 , & 89.3%, respectively compared to plants treated with nematode alone. On the other hand, inoculated plants with *A. chroococcum* one week before nematode inoculation gave the least results in decreasing numbers of galls, egg masses, females / root system and number of J_2 /

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250 g soil by 78, 69, 70 & 88.9% respectively, followed by applying at the same time of nematode inoculation by 77.4, 65, 68.9 & 88.5%, respectively, but when applied one week after nematode inoculation recorded 77, 62, 67.2, & 88.1%, respectively. Also, all treatments significantly decreased the final nematode population and this was reflected on reproduction factor. The least final nematode population obtained with *B. megaterium* when applied one week before

Table (1): Effect of four bacterial strains at three different application times on management of *Meloidogyne* spp. on tomato plants under greenhouse conditions

Bacterial strains	Applications time	Galls/root system	Reduction %	Egg masses/root system	Reduction %	Females/root system	Reduction %	J2/250 g soil	Reduction %	PF	RF
P. fluorescens	One week before	83 ^{def}	83.4	16^{fghi}	84	32.2	82.2	126^{hij}	91.6	257	0.09
	At the same time	87^{def}	82.6	18 ^{efghi}	82	36^{fgh}	80	132^{ghi}	91.2	273	0.09
	One week after	90^{cde}	82	21^{defgh}	79	40 ^{efg}	77.8	141^{fgh}	90.6	292	0.1
A. brasilense	One week before	92^{cde}	81.6	23^{def}	77	44^{def}	75.6	147^{efg}	90.2	306	0.1
	At the same time	100^{bcd}	80	25 ^{def}	75	48^{cde}	73.3	154^{def}	89.7	327	0.1
	One week after	105^{bc}	79	28^{cde}	72	51^{bcd}	71.7	160^{cde}	89.3	344	0.1
A.chroococcum	One week before	110^{b}	78	31^{bcd}	69	54^{bc}	70	167^{bcd}	88.9	362	0.1
	At the same time	113^{b}	77.4	35^{bc}	65	56^{bc}	68.9	172^{bc}	88.5	376	0.1
	One week after	115^{b}	77	38^{b}	62	59^{b}	67.2	179^{b}	88.1	391	0.1
B.megaterium	One week before	70^{f}	86	12^{hi}	88	20^{j}	88.9	100^{k}	93.3	202	0.07
	At the same time	75^{ef}	85	10^{i}	90	25^{ij}	86.1	112^{jk}	92.5	222	0.07
	One week after	79^{ef}	84.2	14^{ghi}	86	29^{hi}	83.9	120^{ij}	92	242	0.08
Nematode alone	-----	500^{a}	----	100^{a}	-----	180^{a}	----	1500^{a}	----	2280	0.8
LSD at 0.05	-----	12.1	-----	7	-----	7.2	-----	31	----		

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Growth characteristics of tomato plants

Data represented in Table (2) showed that all bacterial strains improved growth characters of nematode infected tomato plants compared to plants infected with nematode alone. The best performing conditions are those when bacteria were added one week before nematode inoculation .The treatment of *B.megaterium* achieved the highest increases in shoot and root lengths, shoot and root fresh weights when applied one week before nematode inoculation as recorded 213.3, 175, 180 & 291.3%, respectively. Inoculation tomato plants with *B. megaterium* at the same time of nematode inoculation also enhanced the growth parameters by 200, 162.5, 160 & 269.6%, respectively compared to plants treated with nematode alone. Inoculation tomato plants with *B. megaterium* one week after nematode inoculation recorded low increase percentages in shoot and root lengths as well as weights 193.3, 143.8, 155,175&247.8%, respectively compared to the other application times. Plants inoculated with *P. fluorescens* achieved increase percentages in growth parameters which occupies the second rank after *B.megaterium*, followed by *A. brasilense* and *A.chroococcum*. On the other hand plants inoculated with *P. fluorescens* one week before nematode inoculation augmented the growth parameters such as shoot and root lengths by 173.3 and 131.3 %, respectively as well as shoot and root fresh weights by 140 and 217.4%, respectively, while the shoot dry weight by 162.5%. The treatment of *A. brasilense* occupied the third rank after *P. fluorescens* in increasing the plant growth parameters. The highest percentage of increase in shoots and root lengths by 126.7 and 100 %, respectively. The minimum impact on growth criteria recorded by *A. chroococcum* compared to the other isolates.

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Table (2): Effect of four bacterial strains at three different applications times on plant growth characteristics of tomato plants infected with *Meloidogyne* spp. under greenhouse conditions

Bacterial strains	Applications time	Shoot length(cm)	Increase %	Root length(cm)	Increase%	Shoot fresh weight(g)	Increase %	Root fresh weight(g)	Increase %	Shoot dry weight(g)	Increase %
P. fluorescens	One week before	41^{abc}	173.3	18.5^{abcd}	131.3	24^{abcd}	140	7.3^{abcd}	217.4	10.5^{abc}	162.5
	At the same time	39^{bc}	160	17.6^{abcde}	120	23^{abcde}	130	7^{abcd}	204.3	10^{abcd}	150
	One week after	36^{cd}	140	17^{abcde}	112.5	22^{bcdef}	120	6.4^{abcde}	178.3	9.3^{abcd}	132.5
A. brasilense	One week before	34^{cde}	126.7	16^{abcde}	100	20.5^{cdefg}	105	5.9^{bcde}	156.5	9^{abcd}	125
	At the same time	31^{def}	106.7	15^{abcdef}	87.5	20^{defg}	100	5.4^{cdef}	134.8	8.3^{abcde}	107.5
	One week after	28^{efg}	86.7	14^{bcdef}	75	19^{defg}	90	5^{defg}	117.4	7.8^{bcde}	95
A.chroococcum	One week before	27^{efgh}	80	13^{cdef}	62.5	18^{efg}	80	45^{defg}	95.7	7.1^{cdef}	77.5
	At the same time	25^{fgh}	66.7	12^{cdef}	50	17.3^{fgh}	73	4^{efg}	73.9	6.8^{def}	70
	One week after	22^{gh}	46.7	11^{def}	37.5	16.2^{gh}	62	3.7 ^{efg}	60.9	6.6^{def}	65
B.megaterium	One week before	47^{a}	213.3	22^{a}	175	28^{a}	180	9^{a}	291.3	11.7^{a}	192.5
	At the same time	45^{ab}	200	21^{ab}	162.5	26^{ab}	160	8.5^{ab}	269.6	11.2^{ab}	180
	One week after	44^{ab}	193.3	19.5 ^{abc}	143.8	25.5^{abc}	155	8^{abc}	247.8	11^{ab}	175
Control	-----	20^{hi}	33.3	10^{ef}	25	13^{hi}	30	3^{fg}	30.4	5.4^{ef}	35
Nematode alone		15^{i}		8^{f}		10^{i}		2.3^{g}		4^{f}	
LSD at 0.05	-----	5.6	……	4.7	.....	3.7	-----	1.8	----	2.3	……

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Photosynthetic pigments in shoots of tomato plants

Data illustrated in Table (3) proved that all bacterial strains significantly increased the activity of photosynthetic pigments in plants when applied at all three application time compared to treated plants with nematode alone. Inoculation tomato plants with bacterial strains one week before nematode inoculation fulfilled the highest increase in pigments concentration followed by applying them at the same time then at one week after nematode inoculation. Inoculation with *B. megaterium* gave highest ch.a, chl.b, total chlorophyll and carotenoids as recorded 1.0, 0.75,1.75 and 0.78 mg/ g. f. w. , respectively at one week before nematode inoculation after 30 days from transplanting , while it recoded 2, 1.5, 3.5 and1.8 mg/ g.f.w. , respectively in ch.a, chl.b, total chlorophyll and carotenoids after 60 days from transplanting compared to control . Inoculation plants with *A. chroococcum* gave the lowest photosynthetic pigments compared to other strains when it used at one week after it recorded 0.81,0.60, 1.41and 0.78 (mg/ g.f.w.) respectively, in ch.a, chl.b, total chlorophyll and carotenoids after 30 days from sowing while it recorded 1.61,1.27,2.88 and 1.56 (mg/ g.f.w.) respectively, in ch.a, chl.b, total chlorophyll and carotenoids after 60 days from transplanting. Generally, inoculation bacterial strains one week before nematode inoculation gave highest photosynthetic pigments, while when the inoculation was one week after it recorded lowest photosynthetic pigments.

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Table (3): Effect of four liquid bacterial strains at three different application times on photosynthetic pigments concentration of plants infected with

Bacterial strains	Applications time	Chl.a (mg/ g.f.w.)		Chl.b (mg/ g .f .w.)		Total Chlorophyll (mg/ g .f .w.)		Carotenoids (mg/ g.f .w.)	
		30 days	60 days	30 days	60 days	30 days	60 days	30 days	60 days
P. fluorescens	One week before	0.94^{a}	1.87^{a}	0.72^{a}	1.43^{a}	1.66^{a}	3.3^{a}	0.86^{a}	1.72^{a}
	At the same time	0.92^{a}	1.84^{a}	0.71^{a}	1.41^{a}	1.63^{a}	3.25^{ab}	0.85^{a}	1.7^{a}
	One week after	0.91^{a}	1.82^{a}	0.69^{ab}	1.38^{a}	1.6^{a}	3.2^{ab}	0.85^{a}	1.69^{a}
A. brasilense	One week before	0.89^{a}	1.78^{a}	0.68^{ab}	1.36^{a}	1.57^{ab}	3.14^{ab}	0.84^{a}	1.67^{a}
	At the same time	0.88^{a}	1.76^{a}	0.67^{ab}	1.34^{a}	1.55^{ab}	3.1^{ab}	0.83^{a}	1.65^{a}
	One week after	0.87^{a}	1.73^{a}	0.66^{ab}	1.32^{a}	1.53^{ab}	3.05^{ab}	0.81^{a}	1.62^{a}
A.chroococcu	One week before	0.85^{a}	1.7^{a}	0.65^{abc}	1.3^{a}	1.5^{ab}	3.0^{ab}	0.8^{a}	1.6^{a}
	At the same time	0.83^{a}	1.65^{a}	0.64^{abc}	1.29^{a}	1.47^{ab}	2.94^{ab}	0.79^{a}	1.58^{a}
	One week after	0.81^{a}	1.61^{a}	0.6^{abc}	1.27^{a}	1.41^{ab}	2.88^{ab}	0.78^{a}	1.56^{a}
B.megaterium	One week before	1.0^{a}	2^{a}	0.75^{a}	1.5^{a}	1.75^{a}	3.5^{a}	0.9^{a}	1.8^{a}
	At the same time	0.98^{a}	1.95^{a}	0.74^{a}	1.48^{a}	1.72^{a}	3.43^{a}	0.89^{a}	1.78a
	One week after	0.96^{a}	1.92^{a}	0.73^{a}	1.45^{a}	1.69^{a}	3.37 ^{a}	0.88^{a}	1.75^{a}
Control		0.75^{ab}	1.5^{a}	0.5^{bc}	1.0^{a}	1.25^{bc}	2.5^{ab}	0.6^{b}	1.2^{ab}
Nematode alone		0.6^{b}	1.2^{a}	0.48^{c}	0.95^{a}	1.08^{c}	2.15^{b}	0.5^{b}	0.99^{b}
LSD at 0.05		0.16	0.47	0.12	0.41	0.21	0.66	0.14	0.39

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***Meloidogyne* spp. under greenhouse conditions**

Antioxidant Enzyme in leaves of tomato plants

Data in Figure (3) showed that the activity of certain biological processes was enhanced as a result of using bacterial strains which reflected as inducers for the systemic resistance of tomato plants and bio-control agents on nematode. Peroxidase (POX) and polyphenol oxidase (PPO) were increased by all bacterial strains treatments. Plants treated with different selected bacterial strains exhibited increment in their Peroxidase (POX). The enzymes values were ranged from (0.83 to1.15 µg/g.f wt), the maximum POX activity was induced by *B. megaterium* (1.19 µg/g.f wt) at the same time, followed by (1.15 µg/g.f wt) at one week before. The minimum POX activity was induced by *A. chroococcum* at one week before infected by *Meloidogyne* spp. it recorded (0.86 µg/g.f wt.). Enzyme activity of POX indicated that *B. megaterium* the highest between all treatments followed by *P. fluorescens* > *A.brasilense* > *A. chroococcum*. On the other hand, plants treated with the different selected bacterial strains showed increment in their PPO. The enzymes values were ranged from 0.83 to 1.23 (µg/g.f wt.). The highest increase in PPO enzymes obtained with *B. megaterium* at the same time as it recorded 1.23 (µg/g.f wt.), while the minimum PPO recorded (0.83µg/g.f wt.) with *A. chroococcum* at one week after infected by *Meloidogyne* spp. The presence of *Meloidogyne* spp. only led to increase the POX and PPO (0.3 and 0.4 µg/g.f wt.), respectively compared to healthy plant as recorded (0.6 µg/g.f. wt.)

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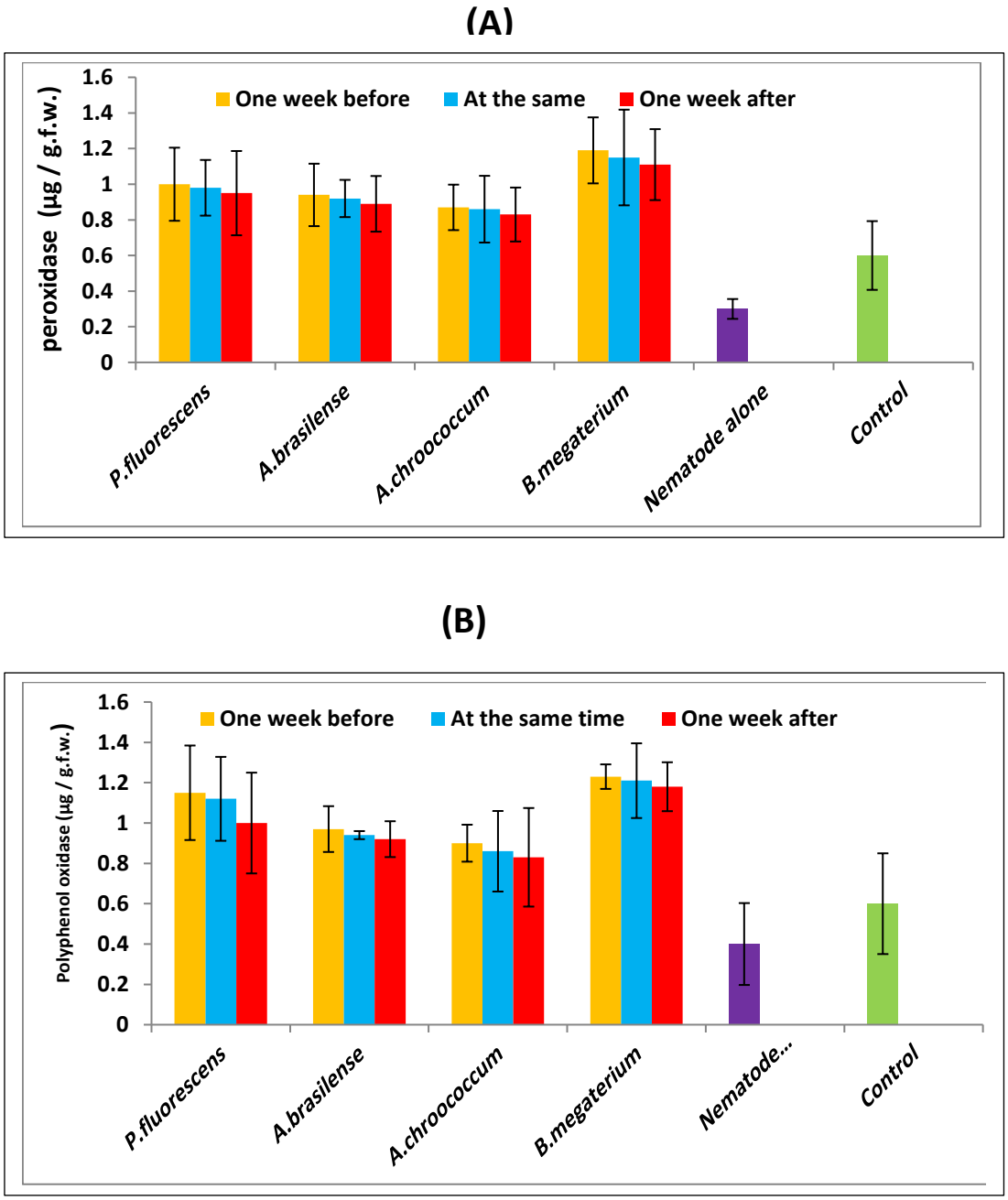

Figure (3): Peroxidase (A) and Polyphenol oxidase (B) in leaves of nematode infected plants inoculated with four liquid bacterial strains at three different application times under greenhouse conditions. Error bars represent the standard deviation between 3.

Enzymatic Activities and total count of bacteria

The enzymes activity in rhizospheric area of plants and total count of bacteria are administered by the activity of beneficial microorganisms

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that colonize the plant roots. Data illustrated in Table (4) proved that there was a significantly increase of dehydrogenase activity and total count of bacteria in all different bacterial strains when they applied at all three different application times compared to plants infected with *Meloidogyne* spp. only. Significant increases in the enzymes activity were attained with the plants inoculated with the microorganisms including; *P. fluorescens, A. brasilense, A. chroococcum* and *B. megaterium* than un-inoculated plants. Applying the bacterial strains at one week before of nematode infected fulfilled the highest enzymatic activity. The enzyme values were ranged from 12 to 21 and 24 to 36 (µg TPF/g soil/day) after 30 and 60 days from transplanting. *B. megaterium* exhibited the highest enzyme activity of dehydrogenase as recorded 21 and 36 µg TPF/g soil/day, respectively after 30 and 60 days at one week before nematode inoculation compared to plants treated with nematode alone. Minimum dehydrogenase activity was recorded by *A. chroococcum* applied at one week after nematode inoculation by 12.0 µg TPF/g soil/day after 30 days from transplanting.

Also, data illustrated in Table (4) showed an increase in bacterial counts in the rhizosphere region of plants in treatments inoculated with different bacterial than in the rhizosphere region in treatments un-inoculated with different bacterial strains. The highest total bacteria count was noted when *B. megaterium* applied at one week before *Meloidogyne* spp. inoculation by $25x10^8$ CFU and $51x10^8$ CFU/g soil after 30 and 60 days from transplanting respectively. Where the lowest bacteria count was noted with *A. chroococcum* when inoculated at one week after nematode inoculation by 5 $x10^8$ and 26 $x10^8$ CFU/g soil, after 30 and 60 days from transplanting respectively compared to other treatments. Additionally, the population of bacteria was increased after 60 days from

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transplanting; on the other hand the total count of bacteria in treated plants with nematode alone recorded lowest bacterial count.

Table (4): Effect of four different bacterial strains at three different applications times on dehydrogenase activity and total count of bacteria of tomato plants infected with *Meloidogyne* spp. under greenhouse conditions

Bacterial strains	Applications time	Dehydrogenase enzyme (µg TPF/g soil/ day)		Total bacterial counts (x10^8 CFU g^{-1} soil)	
		30 days	60 days	30 days	60 days
P. fluorescens	One week before	18.4^{abcd}	29.9^{cd}	19^{bcd}	45^{abc}
	At the same time	18^{bcde}	29^{cde}	17^{cde}	43^{bc}
	One week after	17.7^{bcde}	28.4^{de}	15^{def}	40^{cd}
A.brasilense	One week before	16.5^{cdef}	27.6^{de}	14^{def}	37^{de}
	At the same time	16^{def}	27.2^{ef}	12^{efg}	35^{def}
	One week after	15.2^{efg}	26.8^{ef}	10^{fgh}	33^{ef}
A. chroococcum	One week before	14.4^{fgh}	25^{fg}	8^{ghi}	31^{efg}
	At the same time	13.2^{ghi}	24.8^{fg}	6^{hij}	29^{fg}
	One week after	12^{hij}	24^{g}	5^{hij}	26^{g}
B. megaterium	One week before	21^{a}	36^{a}	25^{a}	51^{a}
	At the same time	20.2^{ab}	34^{b}	23^{ab}	49^{ab}
	One week after	19.3^{abc}	31^{c}	21^{abc}	47^{ab}
Control		11^{ij}	18^{h}	3^{ij}	5^{h}
Nematode alone		10^{j}	15^{i}	2^{j}	4^{h}
LSD at 0.05		2.1	1.9	3.8	4.6

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DISCUSSION

Management of plant disease instigated by parasitic organisms has become a challenging mission to the researchers for sustainable agriculture. More than, 4000 parasitic organisms have been recognized and they can be found in most major biomes. Plant parasitic organisms, absorbs the water and nutrient content from their host plants from the vascular tissues **(Press and Phoenix, 2005**). In this study, we focused on the plant growth promoting rhizobacteria (PGPR) for nematodes bio-control. Results in (Figure 3 and 4) found that all evaluated rhizobacteria viz, (*P .fluorescens, A. brasilense, A. chroococcum* and *B . megaterium*) significantly reduced *Meloidogyne* spp. egg hatching and juveniles mortality compared to control. These results also are agreement with those obtained by **Huang *et al*. (2010)** as they revealed that the bacterial culture of *B.megaterium* YFM3.25 significantly inhibited *M.incognita* egg hatching and juveniles mortality under *in vitro* conditions as well as exhibiting a significant reduction in number of galls, egg masses & eggs /individual egg mass compared to the control under *in vivo* conditions. This bacterium could decrease nematode parameters because it secreted 17 kinds of nematicidal volatiles such as (benzeneacetaldehyde, 2-nonanone, decanal, 2-undecanone & dimethyl disulphide) which were effective against both of juveniles and eggs at the concentration of 0.5 µMol. Other volatiles viz,(phenyl ethanone, nonane, phenol, 3,5-dimethoxy toluene, 2,3-dimethyl-butanedinitrile & 1-ethenyl-4-methoxy-benzene) which have nematicidal effect at proportion of 30-63 %. Six volatiles i.e. (benzene ethanol, propanone, hexadecane, 2-pentylfuran, propyl –benzene & 2,6,10 – trimethyl- dodecane)had a low effect against nematode at the rate of 10%. Also **Aballay *et al*. (2013)** reported that the inhibition of egg hatching was attributed to the secondary metabolites

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produced by the rhizobacteria which caused egg lysis and affect the egg viability. In order to assist if these plant growth promoting rhizobacteria have a biological control for *Meloidogyne* spp. on tomato plants, an experimental setups was used including four PGPR viz (*P. fluorescens, A. brasilense, A. chroococcum & B. megaterium*) against root-knot nematodes under greenhouse condition at three application times (one week before, at the same time and one week after) (Table 1). The time and rate of applications are very significant for the efficacy of bio-control agents. In the present study, repeated applications with one week before showed improved performance than did those applied one week after and at the same time **(Khyami-Horani and Al-Banna, 2006)**. According to **Silveira and Freitas (2007),** the inoculation of the microorganisms in soil must be as early as possible, as the dynamics of the ecosystem that they challenge to invade may hinder their establishment. Inoculation tomato with different bacterial strains had the potentiality decrease in *Meloidogyne* spp. parameters i.e. (galls, egg masses, females/root system and J_2 in soil) and the reproduction to a huge extent compared to treatments treated with nematode alone (Table 1), Liquid of *P. fluorescens* at one week after nematode infected gave reduction percentages in number of galls, egg masses, and females in root system and J_2 in soil. A similar result was reported with those recorded by **Oliveira *et al.* (2009)** they confirmed that *B.megaterium* strains produced secondary metabolites which caused a significant reduction in *Meloidogyne exigua* reproduction on coffee. **Youssef *et al.* (2017)** stated that the rhizobacteria that belong to *Bacillus* i.e. *B. subtilis, B. megaterium & B. pumilus* showed the nematicidal activity against *M. incognita* in addition to ameliorate the growth parameters of sugar beet also, **Sansinenea and Ortiz (2011)**, indicated that *Bacillus* spp. produce

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some substances i.e. antimicrobial compounds (antibiotics) such as zwittermicin which produced by *B.thuringiensis* & *B. cereus* not only served which produced by *B.thuringiensis* & *B. cereus* not only served as an antibiotic and antifungal but also had a nematicidal activity. **Insunza *et al*. (2002)** stated that plant growth promoting rhizobacteria strains (*P.fluorescens, A.chroococcum & A.brasilense*) inhibited egg hatching and killed juveniles through producing a wide variety of antibiotics, siderorhores, hydrolytic enzymes, organic compounds, HCN, phenol oxidation & protease. Interestingly, the four different PGPR showed an effect with all growth parameters measured including shoot length, root length, Shoot fresh weight, Root fresh weigh and dry weight of shoot when applied at one week before of nematode infected (Table 2). *P. fluorescens, A. brasilense, A. chroococcum & B. megaterium* are well known as plant growth promoters **(Resende *et al*., 2004; Fortes *et al*., 2007)**. Different microorganisms such as bacteria could be utilized in biological control arena to protect plants against soil borne pathogens. PGPR bacteria are able to colonize the rhizosphere zone and, therefore, can promote plant health against root-knot nematodes **(Sikora *et al*., 2007).**Plant growth-promoting bacteria could improve plant growth and nutrition, therefore increasing plant resistance against pathogens **(Compant *et al*, 2005 and Liu *et al*., 2012).** Our Results also are agreement with those obtained by **Kalinovskaya *et al*. (2002) ; & Tian *et al.* (2007),**they reported that the suppression mechanism of root-knot nematode created by microorganisms is through parasitism, competition for colonization of sites and nutrients or production of antibiotics such as lipopeptides and surfactin as well as other enzymes and toxins. All crops treated with *B. cereus* S18 combined with *M. incognita* showed plant growth improvement when compared with the bacteria untreated crops

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Mahdy (2002);(Burkett-Cadena *et al.*, 2008). Under greenhouse conditions the plant treated with rhizobacterial strains showed enhanced in photosynthetic pigments comparison to control plants (Table 3) these results are aligned with those reported by **(Abd-El-Khair *et al.* 2019).** They determined that *Bacillus subtilis*, *B. pumilus* and *P. fluorescens* reduced the *M. incognita* spread in the roots of cow pea and improved the levels of photosynthetic pigments. Generally, the four rhizobacterial strains positively impacted ch.a, chl.b, total chlorophyll and carotenoids in tomato plants after 60 days from sowing. PGPR significantly influence the chlorophyll content **(Akhtar *et al.*, 2013; Abo-Koura *et al.*, 2016).**The increasing of chlorophyll in all treatments of PGPR probably resulted in higher photosynthetic rates and thus, improved plants biomass **(Vafadar *et al.*, 2013).** Also, inoculation with bacterial strains significantly increased the carotenoids contents compared to control **(Saharan and Nehra, 2011; Metwally *et al* 2019)**. Another study conducted by **Da Silva *et al.* (2019)** showed that *Pinus pinaster* infested with nematode infection when inoculated with diazotropic bacteria prevented the degradation of plant pigments and improved their synthesis that in turn up regulated the different metabolic activities of the plants photosynthetic activities observed in the present study might be a result of PGPR up regulating the enzymes related with photosynthetic pigments in plants during nematode attack. The similar reduction in the levels of pigments like chlorophyll, carotenoid in plants infected by nematode was revealed earlier by (**Vasil'eva, *et al*., 2003**) where This decline in plant pigments is primarily because of the inhibition of crucial enzymes required in the Violoxanthin pathway, this in turn impairs the stability of the photosynthetic pathway. The data obtained strongly indicate that PGPR has a great potential as a biological product to enhance antioxidant

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activity in shoots of tomato plants (Figure 3).Commonly related with wounds is necessary to activations of biological defense mechanism. Increase in polyphenol phenol oxidase (PPO) and peroxidase activity (POX) appears to be result of an adaptive response which provides the plant with defense against biotic and abiotic stress **(Guida *et al*., 1992).**The protective activity of PPO, and peroxidase (POX) was enhanced in susceptible but decreased in resistant plants **(Zacheo *et al*., 1993).** Peroxidase avoids the deleterious effect of O^{-}_{2} radicals in root cells and transforms it to H_2O_2 which is then transformed by catalase to harmless O_2 + H_2O. Therefore, in susceptible tomato roots infested with *M. incognita* APX activity considerably increased in comparison to uninfected controls and decreased in resistant cultivars **(Zacheo *et al*., 1987)**. These findings are in harmony with our results whereas, catalase (CAT) increased after nematode infection. This effective role of the total phenols (Figure 3) was studied since **1959** where **Clark *et al.***, related the mechanism of disease resistance to the phenolic compounds. They added that this activity because of the quinic acid or caffeic acid parts of chlorogenic acid which are released by the achievement of hydrolytic enzymes such as esterase's. Also, certain phenolic compounds like acetylenes, terpenoid aldehydes, sesquiterpenoids and phenoxypropionic acid derivatives are known to have nematicidal activity **(Veech, 1979; Mori *et al*., 1982; Hayashi *et al*., 1983).**

Enzyme activities could be considered effective indicators of soil quality variations resulting from environmental stress or management practices. Results proved that there was a significantly increase of dehydrogenase activity in all different bacterial strains and total count of bacteria (Table 4). Enzyme activity in soil results from the activity of accumulated enzymes and from enzymatic activity of proliferating

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microorganisms **(Kumar *et al.*, 2016).** Dehydrogenase activity is considered as display of an overall biological activity in the soil **(Benitez *et al.*, 2006; Moeskops *et al.*, 2010).** In line with our expectation, dehydrogenase activity was reduced significantly in treatments infected with nematode only. Reduction in dehydrogenase activity indicates the death or depression of the microbial biomass in the soil most likely as a result of the indirect effect of irradiation. It has also found that measurement of changes in soil enzyme activities may provide a beneficial index of changes in soil quality **(Visser and Parkinson 1992)**.In previous experiments, a significant positive correlation was found between total bacterial count and dehydrogenase activity**(Buchan *et al.*, 2013; Gebremikael *et al.*, 2014b)**, plant roots release wide diversity of compounds such as amino acids, organic acids, oligosaccharides, sugar, vitamins, nucleotides, flavonoids, enzymes, hormones, volatile compounds, phenolics, mucilage, carbohydrates and various secondary metabolites, which are increase biological activities in rhizospheric area **(Rohrbacher and St-Arnaud, 2016).**

CONCLUSION

The current study illustrates the importance of PGPR in vitro conditions for egg hatching inhibition and larvae mortality of *Meloidogyne* spp and their evaluation in controlled conditions in a tomato pots trial. Traits PGPR could prove effective on management of *Meloidogyne* spp, enhancing the growth in the stimulation of plant root system and significant increase in biochemical constitutions and plant defenses. Further studies are needed using new species of PGPR to improve the potentials of bio-agents and succeed safe and ecofriendly management of root-knot nematodes under greenhouse and field conditions.

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