International Journal of Microbiological Research 10 (3): 148-157, 2019
ISSN 2079-2093

DOI: 10.5829/idosi.ijmr.2019.148.157

Effect of Some Bio-Fertilizers on the Root-Knot Nematode *Meloidogyne incognita* Infecting Common Bean (*Phaseolus vulgaris* L.)

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Abstract: Greenhouse and field experiments were conducted to assess the efficacy of *Rhizobium leguminosarum* bv. phaseoli, *Paenibacillus polymyxa* and a mixture of *Trichoderma harzianum* and *Trichoderma viride* as plant growth promoting rhizobacteria (PGPR) combined with composted cattle manure for controlling root-knot nematode, *Meloidogyne incognita* infecting common bean. *Paenibacillus polymyxa* was the superior for phosphate solubilization, ammonia as well as siderophores, protease and hydrogen cyanide production. While, the mixture of *T. harzianum* and *T. viride* appeared to be superior to indole acetic acid (IAA) production. The experiments include adding bio-fertilizers once and twice whether under greenhouse or field conditions. Adding bio-fertilizers once under greenhouse conditions revealed that all treatments significantly ($P \leq 0.05$) reduced the root-knot nematode reproduction during the growing season of common bean compared to control treatment. The nematicide Vydate® (Oxamyl) 24% L treatment had the maximum effect on reducing the number of galls/ root system. While, the highest reduction percentage in root galling was associated with *Paenibacillus polymyxa*, followed by the mixture of *T. harzianum* and *T. viride*. The effect of bio-fertilizers with composted cattle manure, which added twice under greenhouse conditions (one week before and after root-knot nematode, inoculation) was significantly effective for nematode reproduction. The number of galls, egg-masses per root system, number of eggs/egg-mass and finally nematode population in the soil of *M. incognita* was decreased significantly compared to control treatment. While under field conditions, the effect *Paenibacillus polymyxa* with composted cattle manure gave the highest reduction percentage of root galling and egg-masses per root system compared to the control treatment. *Paenibacillus polymyxa* that was added twice was the most efficient in reducing root galling and egg-masses per root system compared to the control treatment. Generally, all the applied bio-fertilizers added once or twice with composted cattle manure significantly increased the plant growth parameters *viz*., shoot length, shoot weight, root weight, root length, number of branches per plant and the number of pods per plant.

Key words: Biological control · *Phaseolus vulgaris* · *Meloidogyne incognita* · Bio-fertilizers · Plant growth promoting rhizobacteria

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important crop worldwide and is widely recommended due to its nutritional components in many countries. It is the main source of vital nutrients, proteins, vitamins and minerals [1]. Globally, 17 million tons of green and dry beans are produced annually [2].

Many plant-parasitic nematodes have been recorded to damage the crop, with a worldwide annual yield loss of around 11% [3, 4]. Among nematode pests, the root-knot nematodes *Meloidogyne* spp., are most pathogens of common bean, where *Meloidogyne incognita* and *M. javanica,* are the most commonly identified as common bean nematodes. They are considered to be one of the main causes of low yield,

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particularly in sandy soils with high temperatures. The losses caused by *M. incognita* and *M. javanica* in common bean crops can reach to 90%.

The application of chemicals can control the plant-parasitic nematodes, but the continued chemical application may have a harmful effect on non-target species and increase soil toxicity. The use of biological control agents is an alternative to chemical control [5].

Biological control is considered as one of the possible safe alternatives for disease management to pesticides and is likely to be free from toxic residual effects. Plant growth promoting rhizobacteria (PGPRs) have beneficial effects on seed germination, emergence and colonization of roots, mineral nutrition and suppression of plant diseases [6]. Mechanisms of PGPR are either directly, indirectly or both. Direct mechanisms include assisting in the uptake of essential nutrients and secretion of plant growth promoting metabolites like indole acetic acid (IAA). Indirect mechanism is through production of substances to reduce or prevent pathogenic effects, such as siderophores and hydrogen cyanide [7].

Several researchers recorded plant-beneficial bacteria and fungi that live in the soil as free organisms or as endophytes that cause plant growth and protect plants against diseases and abiotic factors [8, 9]. The use of *Bacillus* spp., as bio-fertilizers to the soil may increase the plant-available sources of nutrients in the rhizosphere, control the pathogenic microbial growth that causing disease and induce pest defense systems [10, 11]. The fungi, *Trichoderma* spp.,are commonly used for nematode control that can live in soil in the presence of compost around the rhizosphere and have a high nematicidal properties against the plant parasitic nematodes [12]. According to Shebani and Hadavi [13], there are two potential mechanisms for the suppression of nematodes by *Trichoderma* spp., direct egg parasitism by increasing extracellular chitinase activity as demonstrated by the ability to infect eggs and the induction of plant defense mechanisms leading to systemic resistance.

Greenhouse and field experiments were carried out to investigate the effects of the bio-fertilizers as *Rhizobium leguminosarum* (bv. phaseoli), *Paenibacillus polymyxa* and the mixture of (*T. harzianum* and *T. viride*) as bio-control agents in the presence of composted cattle manure, against the root-knot nematode, *Meloidogyne incognita* on common bean. As well as, studying their mechanisms as plant growth promoting substances.

MATERIALS AND METHODS

Microbial and Cattle Manure Source: The plant growth promoting bacteria (PGPB) (*Rhizobium leguminosarum* bv. Phaseoli and *Paenibacillus polymyxa* were used as bio-fertilizers, the mixture of *T.harzianum* and *T. viride* used as plant growth promoting fungi (PGPF) and composted cattle manure were obtained from the Bio-fertilizers Production Unit of Agric. Microbiol. Res. Dept., (SWERI), (ARC), Giza, Egypt.

Microbial Preparation: Vegetative cells of each bacterium were obtained by culturing *Rhizobium leguminosarum* bv. phaseoli on yeast extract mannitol broth medium [14], while nutrient broth medium (Merck, 1450) was prepared for *Paenibacillus polymyxa* cultivation [15]. Cell suspensions of bacterial strains were adjusted to 10^9CFU/ml. *T. harzianum* and *T. viride*, were grown on a potato dextrose agar (PDA) [16] and incubated at 28°C for 4 days on a rotary shaker. The fungi spore density was 1×10^9 spores/ ml.

***In vitro* Screening of Isolates for Plant Growth-Promoting Activities:**

- IAA production was quantitatively measured by the method of Gordon and Weber [17].
- Phosphate solubilization was quantitatively measured according to Pikovskaya [18] and phosphorus content was determined according to Watanabe and Olsen [19].
- Siderophore assay was done by the method of Alexander and Zuberer [20], optical density was measured at 630 nm and siderophores content in the aliquot was calculated by the method of Sayyed *et al*. [21].
- Hydrogen cyanide (HCN) production was determined according to Bakker and Schippers [22].
- Ammonia (NH_3) production was detected by adding 1 ml Nessler's reagent to a 72 h, old culture grown in peptone broth (Peptone- 10g, NaCl-5g, distilled water-1lit) and recorded the presence of the deep yellow to brown color [22].
- Determination of proteolytic activity where the strains were spotted on a skim milk agar plate (containing 0.5 % peptone 0.3 % beef extract, 0.5 % skim milk and 1.5 % agar). The inoculated plates were incubated at 37°C for 24 h. The presence of a clear zone was recorded and used to indicate the bacterial ability to produce proteases [23].

Table 1: Chemical and physical properties of experimental soil.

Property	Soil
Sand (%)	89.79
Silt (%)	2.48
Clay (%)	7.73
Textural class	Sandy
pH	7.32
E.C (dS m^{-1} at 25°C)	0.25
Organic matter (%)	0.25
Organic carbon%	0.15
Total nitrogen (%)	0.021
C/N ratio	7.14

Table 2: Chemical analysis of composted cattle manure

Property	Compost
P^H	7.30
E.C (dS m^{-1} at 25°C)	4.10
Organic matter (%)	28.60
Organic carbon%	16.60
Total nitrogen (%)	0.95
C/N ratio	17.47
Total phosphours (%)	0.31
Total potassium (%)	0.27

Greenhouse Experiment: The experiment was conducted at Ismailia Agricultural Research Station during winter season of 2019. Compost cattle manure, was applied as organic fertilizer kindly supplied by Ismailia Agricultural Research Station, Egypt. The main characters of used soil and compost are shown in Table (1) and Table (2), respectively.

Common bean cultivar Paulista was obtained from the Vegetable Crop Research Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt.

Culturing of Nematode: The pure culture of *Meloidogyne incognita* from single egg-mass has been maintained on tomato seedling (*Lycopersion esculantum* Mill cv. G.S) at 25 ±2°C in order to obtain sufficient numbers of second stage juveniles.

The effect of different bio-fertilizers (*R. leguminosarum* bv.phaseoli, *P. polymyxa* and a mixture of *T. harzianum* and *T. viride*) with composted cattle manure on *M. incognita* reproduction and common bean plant growth parameters under greenhouse conditions was studied.

Five days before sowing, composted cattle manure (50 g/pot), was added to the clay pots, 30 cm in diameter (4 kg soil), filled with a sterilized mixture of clay and sand (4:1 w/w). Pots were kept moist for the decomposition of the organic manure (composted cattle manure). Pots were sown with five seeds per pot a week after emergence, the seedlings were thinned to one seedling in each pot. The bio-fertilizers, *R. leguminosarum* bv. Phaseoli and *P. polymyxa* were added separately (25 ml/ plant at the concentration of 10^9 cfu). As well as, the mixture of *T. harzianum* and *T. viride* was added (12.5ml/ plant of each fungus at concentration of 1× 10^9 spores/ ml). After one week from soil inoculation with the bio-fertilizers, each seedling (15 days old) was inoculated with the root-knot nematode, *M. incognita,* using approximately 1000 newly hatching second stage juveniles (J_2), the nematode inoculum was introduced into the soil with a pipette through three holes 2 cm from the point of sowing. The holes were covered lightly with soil after the inoculation. Control treatment (without bio control agents or nematicide, inoculated with *M. incognita* as mentioned before). A comparison treatment with the nematicide Vydate (Oxamyl) 24% L, was added at the rate of 4 L/Fed (0. 2 ml /plant) as recommended, after two days from nematode inoculation. All treatments were replicated three times. The pots were arranged in a complete randomized design and plants have been regularly watered.

Treatments Applied Were:

A - *Rhizobium leguminosarum* (bv. phaseoli) + composted cattle manure
B - *Paenibacillus polymyxa*+ composted cattle manure
C - Mixture of *T. harzianum* and *T. viride* + composted cattle manure
D - Nematicide treatment (Vydate (Oxamyl) 24% L)
E - Control treatment (nematode only).

All bio-fertilizers treatments were added twice (one week before and after nematode inoculation).

Field Trail: Afield trail was executed to investigate the effect of different bio-fertilizers added twice, the first after germination and the second after a week combined with composted cattle manure on *Meloidogyne* spp., reproduction and common bean plant growth parameters.

A heavily infested field with the root-knot nematodes, *Meloidogyne* spp*.,* at Ismailia Agriculture Research Station during the winter season 2019 was used for the experiments. The micro plots (1 m^2) were used. The composted cattle manure was incorporated into the top15 cm of the beds of plots at the rate of 1 kg/plot five days before sowing.

The soil was treated with the bio-control agent at sowing time in the root zone at the rate of 250 ml as 10^9 cfu for bacteria and 1×10^9 spores /ml for fungi) of each bio-agent/plot. Vydate (Oxamyl) 24% L as a nematicide was used at 4 L / feedan (recommended dose) was used for comparison. Control treatment without bio agents or nematicide. Each treatment was replicated three plots; each micro plot contains nine plants. Treatments were deployed in a completely randomized design under micro plots conditions. Plants were watered three times weekly.

Experiments terminated after sixty days of nematode inoculation. The growth parameters included shoot and root lengths, shoot fresh and dry weights, root, fresh weight, the number of branches per plant, number of pods per plant and pods weights per plant were recorded. Increasing percentages of total plant fresh weight were calculated in comparison with a control treatment.

Data on the number of galls formed on the whole root system, root gall index (G.I), egg-masses/root system, eggs/egg-mass for three plants of each plot and the second-stage juveniles (J_2) in 250 g soil were also determined for each plot. The number of root galls, root gall index (G.I) and egg- masses index (E.I.), were counted according to Taylor and Sasser [24]. Roots were examined under binocular microscope for counting the number of galls and egg-masses after staining for 15-20 min in an aqueous solution of phloxine B (0.15 g per L) [25]. Reduction percentages of root knot nematode galls, egg-masses numbers/ root system, number of eggs/egg-mass and number of the second-stage juveniles (J_2) in 250 g soil were calculated in comparison with a control treatment. Eggs/egg-mass, egg masses Garcia-Fraile *et al*. [10] from each treatment (all replicates) were randomly selected from the root and each egg mass was crushed using a drop of sodium hypochlorite solution (0.01%). Gelatinous matrix was dissolved and observe under a light microscope [26]. Soil after removing the plant, was mixed well and 250 g of soil from each plot was used to extract nematode, using sieving and Baermann pan technique [27]. The same data were recorded with all plants for each treatment in the experiments under greenhouse conditions.

All experiments were performed twice. Analysis of variance was carried out using MSTAT-C program version 2.10 [28]. Least significant difference (LSD) was employed to test for significant difference between treatments at $P \leq 0.05$ [29].

RESULTS

***In vitro* Screening of Isolates for Plant Growth-promoting Activities:** As shown in Table (3), the microbial strains produced IAA in culture broth in amounts varied significantly among the strains in the order mix of *T. harzianum* and *T. viride Paenibacillus polymyxa R. leguminosarum* bv. Phaseoli. The corresponding amounts were 112.11, 79.03 and 61.18 μgmL^{-1}, respectively.

The greatest phosphate solubilization was induced by *Paenibacillus polymyxa* (123.20 ppm) followed by mixture of *T. harzianum* and *T. viride* (108.70 ppm) and the least by *R. leguminosarum* (89.22 ppm) (Table 3).

The highest quantity of siderophores were produced by *Paenibacillus polymyxa* followed by *R. leguminosarum* bv. phaseoli and mixture of *T. harzianum* and *T. viride* in amounts of 59.18, 22.58 and 14.67 %,respectively (Table 3).

Concerning protease enzyme, the tested strains produced clear zones with diameters ranged between 0.89 and 1.68 cm. The bacterial strain which exhibited the highest activity was *Paenibacillus polymyxa* (Table 3). NH_3 was detected by adding 1 ml Nessler's reagent to broth medium and the presence of the deep yellow to brown color was recorded. *Paenibacillus polymyxa* was the superior for NH_3 production.

The visual inspection of the plates indicated *R. leguminosarum* bv. Phaseoli was the inferior hydrogen cyanide producer (Table 3).

Data presented in Table (4) showed the effects of bio-fertilizers (*Paenibacillus polymyxa, Rhizobium leguminosarum* bv. Phaseoli and the mixture of *Trichoderma harzianum* and *T. viride*) by adding them one week before nematode inoculation in combination with composted cattle manure on the development of *M. incognita* and common bean growth under greenhouse conditions. Results revealed that all treatments had the potential to reduce significantly the root-knot nematode *M. incognita* reproduction during the growing season. The nematicide Vydate® (Oxamyl) 24% L had the maximum effect to reduce the number of root

Table 3: Biological active substances produced by microbial strains

Treatments	IAA ug/ ml	Phosphorous ppm	Protease	NH_3	HCN	Siderophores %
R. leguminosarum bv. Phaseoli	61.18 c	89.22 c	+	+	+	22.58 b
Paenibacillus polymyxa	79.03 b	123.15 a	+++	++	++	59.18 a
Mix of *T. harzianum* and *T.viride*	112.11 a	108.65 b	++	+	++	14.67 c
Control	-	-	-	-	-	-

Different letters indicate significant differences among treatments within the same column according to least significant difference test ($P \leq 0.05$)

Table 4: Efficacy of adding bio-fertilizers once as (one week before nematode inoculation) in combination with composted cattle manure in controlling *M. incognita* on common bean plants under greenhouse conditions

Treatments	No. Galls/ root	Red (%)	No. Egg -mass/ root	Red (%)	No. Eggs/ egg-mass	Red (%)	No. J_2/ 250 g soil	Red (%)	Gall index	Egg-mass index
R. leguminosarum bv. Phaseoli	109 b	60	51.6 b	62	217.6 b	52	220c	71	5	4
Paenibacillus. polymyxa	67.6 bc	75	40 b	71	185.3bc	59	180 d	76	4	4
T. harzianum + *T. viride*	76.6 bc	72	50.3b	63	210 b	53	380 b	49	4	4
Vydate®	36 c	87	18.6c	86	171 c	62	120 e	84	4	3
Control	274.6 a	00	136.3a	00	449 a	00	746.6a	00	5	5

Different letters indicate significant differences among treatments within the same column according to least significant difference test ($P \leq 0.05$). *Root gall index (GI) or egg-masses index (EI) : 0= none, 1=1-2, 2= 3-10, 3=11-30, 4=31-100 and 5 =more than 100 galls per root system

Table 5: Efficacy of adding bio-fertilizers once as (one week before nematode inoculation) in combination with composted cattle manure on plant growth parameters of common bean plants under greenhouse conditions

Treatments	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	No. Branches/ plant	No. Pods/ plant	Pods weight/ plant (g)	Total plant fresh weight (g)	Ice (%)	Dry shoot weight (g)
R. leguminosarum bv. Phaseoli	25.6 ab	11.3ab	30a	5.8 b	10.6 a	2 b	8 bc	17.1	84	5b
Paenibacillus polymyxa	28.6 a	14a	29 a	9.3 a	11.3 a	6 a	13.3 a	23.3	151	9a
T. harzianum+*T.viride*	24 ab	11ab	28.6a	5b	10.6 a	5.3 a	9.6b	16	72	7.6a
Vydate®	21b	8.7b	28a	5.2b	7a	2.3 b	5.6 c	13.9	49	4b
Control	15.3c	5c	23b	4.3b	3.6 b	1 b	1.3 d	9.3	00	3.3b

Different letters indicate significant differences among treatments within the same column according to least significant difference test ($P \leq 0.05$)

Table 6: Efficacy of adding bio-fertilizers twice as (one week before and after nematode inoculation) in combination with composted cattle manure in controlling *M. incognita* on common bean plants under greenhouse conditions

Treatments	No. Galls / root	Red (%)	No.Egg-mass/root	Red (%)	No. Eggs/ egg-mass	Red (%)	No. J_2/ 250 g soil	Red (%)	Gall index	Egg-mass Index
R. leguminosarum bv. phaseoli	56.6 b	79	29.6 b	78	213.6 b	52	160 b	79	4	3
Paenibacillus polymyxa	47b	83	13.6 c	90	122 d	73	80 d	89	4	3
T. harzianum+*T.viride*	60 b	78	23.6 bc	83	179.6 bc	60	160 b	79	4	3
Vydate®	36b	87	18.6 bc	86	171c	62	120 c	84	4	3
Control	274.6 a	00	136.3 a	00	449a	00	746.6a	00	5	5

Different letters indicate significant differences among treatments within the same column according to least significant difference test ($P \leq 0.05$). *Root gall index (GI) or egg-masses index (EI) : 0= none, 1=1-2, 2= 3-10, 3=11-30, 4=31-100 and 5 =more than 100 galls per root system

galling by (87%). While, among bio-fertilizes treatments, the most effective percentage reduction in reducing root galling / root system was recorded with *Paenibacillus polymyxa* (75%), followed by the mixture of *T. harzianum* and *T. viride* (72%). Significant reductions in number egg-masses/ root system were observed with Vydate® (Oxamyl) 24% L treatment and *Paenibacillus polymyxa* being 86 and 71% respectively. The number of eggs per egg-mass also significantly reduced, the highest reduction was recorded with Vydate® (Oxamyl) 24% L (62%), followed by *Paenibacillus polymyxa* (59%). The same result was noticed for the number of second stage juvenile J_2 in soil. The gall index (GI) was rated between 4 to 5 for treatments. The egg-mass index (EI) between 3 to 5 for treatments.

Data presented in Table (5) revealed that all bio-fertilizers applied in combination with composted cattle manure significantly increased the plant growth parameters compared to the control treatment. The maximum shoot length (28.6cm), shoot weight (14 g) and root weight (9.3 g), root length (29cm), the number of branches per plant (11.3), number of pods per plant (6), as well as dry shoot weight (9g) was recorded with *Paenibacillus polymyxa*. There were significant increases in the total plant fresh weights (shoot and root) for all treatments compared with control. The highest increases in the total plant fresh weights were recorded with treatment *Paenibacillus polymyxa* (151%), followed by *R. leguminosarum* bv. phaseoli (84%).

Data presented in Table (6) show the effects of *Paenibacillus polymyxa, R. leguminosarum* bv. phaseoli and the mixture of *T. harzianum* and *T. viride* in combination with composted cattle manure, which added twice (one week before and after nematodeinoculation) on *M. incognita* reproduction. The numbers of galls, egg-masses per root system, numbers of eggs/egg-mass and finally nematode population in soil of *M. incognita* decreased significantly with the application of treatments compared to a control treatment. The most effective treatment in reducing the root galling the nematicide Vydate® (Oxamyl) 24% L at a rate of percentage of reduction (87%). While, *Paenibacillus polymyxa* was effective in reducing the number of egg-masses/ root

Table 7: Efficacy of adding bio-fertilizers twice as (one week before and after nematode inoculation) in combination with composted cattle manure on plant growth parameters of common bean under greenhouse conditions

Treatments	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	No. Branches/ plant	No. Pods/ plant	Pods weight/ plant(g)	Total plant fresh weight (g)	Inc (%)	Dry shoot weight (g)
R. leguminosarum bv. phaseoli	30.6 b	16.6 b	38 a	9 b	14 a	7 a	15 a	25.6	175	12 b
Paenibacillus polymyxa	36 a	21.6 a	40 a	11.3 a	14a	8 a	17 a	32.9	254	16 a
T. harzianum, +*T.viride*	26.6 b	14.3 b	36 a	8b	12.5a	3.6 b	12 a	22.3	140	9.5 b
Vydate®	21c	8.7c	28 b	5.2c	7b	2.3 bc	5.6 b	13.9	49	4 c
Control	15.3 d	5d	23 b	4.3c	3.6b	1 c	1.3 b	9.3	00	3.3 c

Different letters indicate significant differences among treatments within the same column according to least significant difference test ($P \leq 0.05$).

Table 8: Efficacy of adding bio-fertilizers once as (at sowing time) in combination with composted cattle manure on the root-knot nematodes, *Meloidogyne* spp., reproduction on common bean under field conditions.

Treatments	No. Galls / root	Red (%)	No.Egg-mass/ Root	Red (%)	No. Eggs/ egg-mass	Red (%)	No. J_2/ 250 g soil	Red (%)	Gall index	Egg-mass index
R. leguminosarum bv. Phaseoli	180b	29	100 b	29	273 b	40	360 b	57	5	5
Paenibacillus polymyxa	144c	43	75 c	46	250 b	45	266.6 c	68	5	4
T. harzianum, + *T. viride*	159bc	37	91 b	35	234b	48	266.6 c	68	5	4
Vydate®	61d	76	31 d	78	151c	67	86.6 d	90	4	4
Control	253a	00	140a	00	454a	00	840 a	00	5	5

Different letters indicate significant differences among treatments within the same column according to least significant difference test ($P \leq 0.05$). *Root gall index (GI) or egg-masses index (EI) : 0= none, 1=1-2, 2= 3-10, 3=11-30, 4=31-100 and 5 =more than 100 galls per root system.

system, the highest percentage of reduction was (90%) and the number of eggs/egg-mass with a percentage of reduction was (73%). The same result was recorded for the number of second stage juvenile (j_2) with percentage of reduction (89%). In case of root gall index, the results were recorded as appointed in 4 for the tested bio-fertilizer treatments vs. 5 for the control treatment. The egg-masses indices of all tested treatments ranged from 3 for all bio-fertilizer treatments while, the control treatment was 5.

Results in Table (7) revealed that all tested bio-fertilizers that added twice with composted cattle manure obviously enhanced plant growth parameters. The most effective treatment was *Paenibacillus polymyxa*. The maximum shoot length was 36cm, root length was 40cm, branches / plant was 14. Additionally, the highest numbers of pods per plant and pod weights were recorded with *Paenibacillus polymyxa*. The highest increases in total plant fresh weight were (254%) with *Paenibacillus polymyxa*, followed by *R. leguminosarum* bv. Phaseoli (175%). Dry shoot weight also, increased with bio-fertilizer treatments compared with the control treatment.

Under field conditions, two experiments were performed to examine the effect of three different bio-fertilizers in controlling the root-knot nematodes, *Meloidogyne* spp., in common bean plants.

Data in Table (8) present the influence of bio-fertilizers *viz*., (*Paenibacillus polymyxa, R. leguminosarum* bv. phaseoli and the mixture of *T. harzianum* and *T.viride*) in combination with composted cattle manure on the root-knot nematodes, *Meloidogyne* spp., reproduction on field grown common bean. The most effective treatment at the reduction percentage of root galling per root system, eggs/ egg-mass, reduction in egg-masses per root system, as well as, the second stage juvenile in the soil was the nematicide Vydate® (Oxamyl) 24% L. Among the bio-fertilizers the treatment, *Paenibacillus polymyxa* gave the highest reduction percentages in root galling and egg-masses per root system by (43%) and (46%) respectively. The highest reduction percentage in the number of eggs/ egg-mass was (48%) with the mixture of *T. harzianum* and *T. viride*. At the same time, the gall index not affected by treatment, it was 5 with all bio-fertilizer and control treatments. No differences were reported between *R. leguminosarum* bv. Phaseoli and control treatment in the egg mass index (5).

The effects of bio-fertilizers on plant growth parameters of common bean infected with the root-knot nematodes, *Meloidogyne* spp.,under field conditions are presented in Table (9). The most efficient treatment was *Paenibacillus polymyxa*, recording the highest shoot length (22.5cm), root length (36cm) and number of branches per plant (13.7). Also, this treatment recorded the highest total plant fresh weight (93%), shoot dry weight (10.3g). On the other hand, the mixture of *T. harzianum* and *T. viride* recorded the highest weights per plant (7g).

Data in Table (10) showed that all treatments that applied twice were efficient in reducing nematode reproduction. Among the bio-fertilizer, *Paenibacillus polymyxa* was the most efficient in the percentage of reducing root galling and egg-masses per root system (56% and 67%) compared to control treatment. The nematicideVydate® (Oxamyl) 24% L was more effective in percentage of reducing in the second stage juveniles in soil with (90%) compared with the control treatment, followed by *Paenibacillus polymyxa* with (86%).

Table 9: Efficacy of adding bio-fertilizers once as (at sowing time) in combination with composted cattle manure on plant growth parameters of common bean under field conditions

Treatments	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	No. Branches/ plant	No. Pods/ plant	Pods weight/ plant (g)	Total plant fresh weight (g)	Inc (%)	Dry shoots weight (g)
R. leguminosarum bv. phaseol*i*	19.3 ab	13ab	32 ab	5.6 a	10.7bc	5.3a	6 ab	18.6	71	9a
Paenipacillus polymyxa	22.6 a	15a	36 a	6 a	13.7a	6a	6.5 ab	21	93	10.3a
*T. harzianum+T.*viride	21 ab	14 a	30 b	6.3a	11.6ab	5.3a	7 a	20.3	86	9.3a
Vydate®	18 ab	11.3 b	23 c	5.3 ab	8.7c	2b	4.5 b	16.6	52	7b
Control	15 b	6.6 c	15 d	4.3 b	5d	1b	4b	10.9	00	4.5c

Different letters indicate significant differences among treatments within the same column according to least significant difference test (P ≤ 0.05)

Table 10: Efficacy of adding bio-fertilizers twice as (the first one at sowing time and the second a week later) in combination with composted cattle manure on the root-knot nematodes, *Meloidogyne* spp., reproduction, on common bean plants under field conditions

Treatments	No. Galls / root	Red (%)	No.Egg mass/root	Red (%)	No. Eggs/ egg-mass	Red (%)	No. J2/ 250 g soil	Red (%)	Gall index	Egg-mass index
*R. leguminosarum*bv. Phaseoli	153.3 b	39	57.6 b	59	244 b	46	233.3b	72	5	4
Paenibacillus polymyxa	111.6 c	56	46.3 bc	67	233 b	49	120 cd	86	5	4
T. harzianum, + *T. viride*	118.3 c	53	62.6 b	55	234 b	48	160 c	81	5	4
Vydate®	61 d	76	31c	78	151 c	67	86.6d	90	4	4
Control	253a	00	140 a	00	454a	00	840 a	00	5	5

Different letters indicate significant differences among treatments within the same column according to least significant difference test (P ≤ 0.05). *Root gall index (GI) or egg-masses index (EI) : 0= none, 1=1-2, 2= 3-10, 3=11-30, 4=31-100 and 5 =more than 100 galls per root system.

Table 11: Efficacy of adding bio-fertilizers twice as (the first one at sowing time and the second a week later) in combination with composted cattle manure on plant growth parameters of common bean plants under field conditions

Treatments	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight(g)	No. Branches/ plant	No. Pods/ plant	Pods weight/ plant (g)	Total plant fresh weight (g)	Inc (%)	Dry shoot weight (g)
R. leguminosarum bv. Phaseoli	30.6a	21.3a	35.3a	9.3 a	13.6 a	9a	9.6a	30.6	180	14.3a
Paenibacillus polymyxa	26 b	23.3a	27.6 b	8.6a	16.3 a	6b	9a	31.9	193	16.3a
T. harizianum + *T. viride*	24.6b	13b	27.3b	6.3b	9.3 b	5b	9.6a	19.3	77	9.6b
Vydate®	18c	11.3bc	23.3b	5.3b	8.6bc	2c	4.3 b	16.6	52	7bc
Control	15c	6.6c	15c	4.3b	5 c	1c	4b	10.9	00	4.5c

Different letters indicate significant differences among treatments within the same column according to least significant difference test (P ≤ 0.05).

Results in Table (11) indicated that all bio-fertilizers tested added twice with the composted cattle manure improved plant growth parameters compared to control treatment. The most efficient treatment was *R. leguminosarum*bv. Phaseoli with the highest shoot length (30.6cm), root length (35.3cm), root weight (9.3g) and number of pods per plant (9). While, the number of branches per plant was the highest with *Paenibacillus polymyx,* as well as, total plant fresh weight (193%).

DISCUSSION

Microbial communities in the rhizosphere and the alternative to chemical fertilizers have become a subject of great interest in sustainable agriculture and the bio-safety programs. In the coming decades, a major focus would be on safe and environmentally friendly methods to exploit beneficial microorganisms in sustainable crop production. In this study, some of the bio-fertilizers that could control nematodes and their characteristics as antagonistic agents were evaluated as well as, their benefit on the growth of common bean plants with infested the root-knot nematode *M. incognita*, under greenhouse and field conditions.

It is well known that plant growth-promoting rhizobacteria as bio-fertilizers influence the growth and crop yield. In the present study *Rhizobium leguminosarum* bv. Phaseoli, *Paenibacillus polymyxa* and the mixture of *Trichoderma harzianum* and *T. viride,* possess direct plant growth promoting activities like, IAA production and phosphate solubilization. Additionally, their indirect mechanisms included suppressing nematode HCN as well as protease, siderophore and ammonia production.

In greenhouse experiments, data indicated significant increases in plant growth parameters. The application of *Paenibacillus polymexa* whether, added once or twice in combination with composted cattle manure increased shoot length, shoot weight, root weight, root length, the number of branches per plant and number of pods per plant. While, the treatment of *R. leguminosarum* bv. phaseoli twice in combination with composted cattle manure under field conditions enhanced the plant growth criteria, these increases may be attributed to the synthesis of plant auxin IAA and phosphate solubization [30, 31].

Results obtained under greenhouse and field conditions indicated that all bio-fertilizer treatments significantly reduced nematode infection in comparison with the control treatment. *Paenibacillus polymyxa* added once or twice in combined with composted cattle manure reduced the root-knot nematode *M. incognita* reproduction. In this context, organic amendments have been proven to be adverse to plant-parasitic nematodes due to release of NH_4, formaldehyde, phenol and volatile fatty acids [32]. It is postulated that the unfavorable impact of the organic amendment on parasitic nematode is attributed to increasing host resistance to nematode infection and enhancement of growth performance [33]. Compost improves cation exchange and provides vitamins, hormones, plant enzymes and humic acid. The effect of humic acid on plant roots infected by root-knot nematodes can reduce the contents of lipid peroxidation and H_2O_2 in plant roots by improving the specific activities of antioxidants enzymes. [34, 35]. The use of the antagonistic fungi and the plant-growth-promoting rhizobacteria combined with composted cattle manure improved controlling root-knot nematode *M. incognit*a in tomato plants under field conditions. The improved as a result of increased antagonistic fungal multiplication and the PGPRs with composted cattle manure in soil, which favors natural enemies [36].

These results are in the line with those reported by Mekete [37] who revealed that *B. pumilus* reduced the number of root-galling and egg-mass of *M. incognita* on *Ethiopian coffee*. This may be due to the ability of *Paenibacillus polymyxa* to produce hydrogen cyanide (HCN), similarly with the results of Abdel-Baset [38] who assessed the potential of *Paenibacillus polymyxa* to produce HCN. Cyanide ion derived from HCN was found to be a potent inhibitor of many metalloenzymes including copper-containing cytochrome *c* oxidases [39]. It contributes to the broad-spectrum antimicrobial activity and suppression of different plant root diseases. Additionally, HCN has been suggested to have detrimental effects on some plant-pathogenic nematodes [40, 41]. It is well established that HCN, being a potent inhibitor of respiratory pathways, can cause hypoxia and paralytic death of nematodes [40]. It has been anticipated that HCN is the main factor in the biological control of the root-knot nematode, *Meloidogyne hapla* [42]. HCN exerts its toxic effects by inhibiting cytochrome c oxidase, the final component of the aerobic respiratory chain, as well as other essential metalloenzymes [43].

As for siderophores production, iron is an essential element for growth and function of most living cells and it is a vital component in a wide variety of biochemical reactions in plants and microorganisms. The ability of different microorganisms and strains to produce siderophores was evidenced by many investigators [44, 45]. Although different bacterial siderophores vary in their ability to sequester iron, generally, they are deprived pathogenic fungi of this essential element since the fungal siderophores have lower affinity. Siderophores are very important compounds in which it's bind and solubilize extra cellular Fe^{3+} which is then available to the microorganisms [46].

Previous studies revealed that microbial proteases can play a major role in the infection of nematodes (host) by degrading the protecting barriers of the host [47]. It was observed also, that bacterial proteases dissolved and digested nematode cuticle or killed [48, 49]. This is may be owing to the cuticle of nematodes is composed of proteins and chitin, especially the outer part that is covered by a layer of proteinaceous membrane, that is an effective barrier protecting nematodes against damage [50]. At the same time, Rodriguez-Kabana [51] mentioned that ammonia produced by ammonifying bacteria from the decomposition of nitrogenous organic materials can result in reducing nematode populations in soil. Ammonia production by the *Trichoderma* isolates may influence plant growth indirectly which is directly or indirectly useful for plants. The ACC (1-aminocyclopropane-1-carboxylic acid) synthesized in plant tissues by ACC synthase is thought to be exuded from plant roots and be taken up by neighboring micro-organisms. *Trichodrema* may hydrolyze ACC to ammonia [52].

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