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Biotrophic interactions between some biocontrol agents and *Meloidogyne* spp. on Eggplants

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ABSTRACT

Four isolates of different bacterial genera *i.e., Bacillus megaterium*, *Pseudomonas fluorescens*, *Azospirillum brasilense* and *Paenibacillus polymexa* in addition to one *Trichoderma harzianum* isolate were tested either *in-vitro* or *in vivo* to determine their antagonistic potential toward root-knot nematodes on eggplants. Results of *in vitro* studies showed that the highest egg hatching and juveniles mortality percentage of *Meloidogyne* spp. were recorded with *B. megaterium*. The lowest egg hatching recorded with the same biocontrol agent *B. megaterium*, under greenhouse conditions. The highest reduction in nematode characters *i.e.,* numbers of galls, egg masses, females/root system and numbers of J_2/250 g soil was achieved with *B. megaterium* by 82.9, 84.9, 84.3 and 82.0%, respectively, especially when plant was inoculated one week prior to nematode inoculation. In the contrary the lowest effect was occurred particularly with *P. polymexa* by 47.0, 52.0, 50.9 and 53.3%, respectively, when bioagents inoculated after 7 days of nematode inoculation compared with those treated with nematode only.

Key words: Eggplant, Root-knot Nematodes, Biocontrol Agents.

INTRODUCTION

Eggplant is one of the top ten vegetables grown in the world. It contains a small quantity of 92.7% water, 1.4% protein and vitamin A (Murslain *et al*., 2014). Nematodes are a major pathogen to vegetable all over the world, and cause severe damage that leads to high losses of yield. Damage in crops is non-specific and causes different symptoms such as wilting, stunting, nutrient deficiency, root lesions, reduced flowering and even death (Sayed *et al.,* 2019; Bakr *et al*., 2020). Nematicides causes' soil and environmental pollution, and farmers are not careful in using these nematicides and other toxic chemicals. Nematicides are more expensive; however, a using alternative natural material was more effective, safe and has several biological activities (El-Ashry *et al*., 2021). The fungal genus of Trichoderma is one of the fast-growing fungi and is widely distributed in soil. Its different species have attracted much attention as biocontrol agents against nematodes. Besides their parasitic

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activities, the different species of Trichoderma produce some nematicide compounds such as acetic acids and produce different enzymes, toxins and secondary metabolites (Javeed *et al.,* 2016). Among the bioagents, plant growth-promoting rhizobacteria (PGPR)-such as *Pseudomonas fluorescens* has a high efficiency in controlling plant pathogens like root-knot nematodes. The outstanding feature of *P. fluorescens* its high solubilization capacity of soil phosphorous (Osman *et al.,* 2011). Some strains of *P. polymyxa* stimulate plant growth via nitrogen, phosphorus and potassium uptake in nutrient-deficient soils and recently have been found to exhibit strong nematicidal and fumigant activity against *M. incognita*. This fumigant effect is of great importance because it helps to reach target nematodes that reside far from nematicides in the soil (Cheng *et al*., 2017). *B. megaterium* is reducing egg hatching and infection rate of *M. incognita* by producing nematicidal compounds such as Benzeneacetaldehyde; 2- nonanone; Decanal; 2-undecanone and dimethyl disulfide (Engelbrecht *et al.,* 2018). *Azospirillum* is one of the best-studied bacterial genera of (PGPR) at present. These bacteria can fix nitrogen and produce several phytohormones, mainly auxins and particularly indole-3-acetic acid (Okon *et al*., 1983).

The present study is aiming to determine, *in vitro* and *in viv*o, the antagonistic capacity of some bioagents against *Meloidogyne* spp.

MATERIALS AND METHODS

Inoculum of *Meloidogyne* spp.:

Ninety days-old nightshade roots infested with root-knot nematodes, *Meloidogyne* spp. were used to extract nematode inoculum. Roots heavily-infested with *Meloidogyne* spp. was gently uprooted and removed the adherent soil particles by washing under running tap water. Roots were cut to small slices and macerated in Warning blender at high speed for two periods of 10 seconds. Highest number of eggs/larvae released from roots by this method. Root solution was placed in a flask containing sodium hypochlorite (NaOCl). The final concentrations of NaOCl adjust to 0.5% by adding water (Hussey and Barker, 1973). The flask was strongly shaken for 3 minutes to release nematode eggs from the gelatin matrix of egg mass. The flask contents of solution was poured through a group of sieves of different sizes to remove the remains of root tissues. Larvae /eggs were collected on the last sieve of 20µm in size, washed with running tap water to get rid the residue of NaOCl. Eggs were collected in a flask containing tap water. Number of eggs/ml was counted in counting dish under a stereomicroscope. Second-stage juveniles were obtained according to trays technique of Oostenbrink (1960). After 72 hours from the trays set up the number of second-stage juveniles/ml was counted in counting dish under a stereomicroscope.

Bacterial and fungal isolates

Bacterial strains were obtained from Agricultural Research Center (ARC), Soils, Water and Environment Research Institute (SWERI), Giza, Egypt. The bacterial strains were plant growth promoting rhizobacteria (PGPR) viz; phosphate solubilizing bacterium (*Bacillus megaterium*), nitrogen-fixing bacteria (*Paenibacillus polymyxa* & *Azospirillum brasilense*) and *Pseudomonas fluorescens*. The bacterial strains routinely saved on nutrient agar medium at 4°C according to Difco Manual (1985).

Bacterial inoculum preparation

Bacterial inoculum was prepared by inoculating a loop of used bacteria grown on nutrient agar medium in a flask contains 100 ml of nutrient broth medium and incubated on a rotary shaker for 24 hours at 28°C at 100 rpm.

Fungal inoculum preparation

T. harzianum fungus inoculum was prepared by inoculating equal disk of fungal culture in a flask contains 100 ml of potato dextrose broth medium. Flasks incubated on a rotary shaker for five days at 25°C at 100 rpm. Mycelium mass was homogenized with medium culture filtrate in a blender and spore suspension counted and adjusted to a concentration of $1x10^4$ spores/ml by Haemicytometer slide.

Laboratory Experiments

Effect of homogenized liquid of bacterial and fungal isolates on egg hatching of *Meloidogyne* spp.

Nine out of ten ml of homogenized liquid of four bacterial and Trichoderma isolates was placed into wells of microtiter plates then each well received one tenth ml water containing 100 eggs of *Meloidogyne* spp. One hundred eggs in one ml tap water served as a control. Three replicates were used for each particular treatment. Effect of bacterial and fungus on egg hatching was determined after 1, 2, 3 and 7 days under the light microscope by calculating the percentage of hatched eggs.

Effect of homogenized liquid of bacterial and fungal isolates on juveniles mortality of *Meloidogyne* spp.

Nine out of ten ml of homogenized liquid of four liquid bacterial and Trichoderma isolates was placed into wells of microtiter plates then each well received one tenth ml water containing 100 freshly hatched larvae of *Meloidogyne* spp. One hundred freshly second stage larvae in one ml tap water served as a control. Each treatment was repeated three times. Microtiter plates were incubated at different intervals times' viz., 1, 2, 3 and 7 days under room temperature. The effect of the bacterial and fungal on juveniles' activity was examined under the microscope. Inactive juveniles appear to be rigid and elongated with head and tail sometimes slightly bent in total. Larvae mortality percentage was calculated as follows:

% of larvae mortality = number of dead larvae in each treatment / total number of larvae x 100.

Greenhouse Experiment:

Effect of different bioagents at three different application times against *Meloidogyne* spp. on eggplant.

The experiment was carried out under greenhouse conditions at the Farm of Faculty of Agric., Menoufia Univ., Shebin El-Kom, Egypt to evaluate the four mentioned biocontrol agents against root-knot nematodes, *Meloidogyne* spp. on eggplants seedlings (Var. 108-3-1). The bacterial and fungal suspensions were added at three different application times: before one week, at the same time and after one week of nematode inoculation.

Four weeks old eggplant seedlings (Var. 108-3-1) were transplanted into pots each pot contain one seedling. Pots (15 cm in diam.) filled with 2 kg mixture of loamy-sandy soil at (1:2; v/v). Ten ml of each bacterial and fungus isolate were inoculated around the seedling roots. Drenching plants with 10 ml tap water was negative control. Pots inoculated with nematode only served as a positive control. Each treatment was replicated three times. Three thousand eggs/larvae per plant were inoculated simultaneously at seedlings transplanting by pipetting into 3-4 holes around the root zone. Plants were irrigated daily and fertilized once a week by receiving 5 ml of 2 g/l (N: P: K, 20:20:20). Nutrient solution was manufactured by International Egypt Company for Agricultural and Industrial Developing. All pots were arranged in a complete randomized block design in a greenhouse as described by Duncan (1955). Sixty days of nematode inoculation, plants were removed; roots were rinsed with running tap water. Recorded nematode parameters are the number of galls, egg masses, females/root system, J_2/250 g soil, final population (Pf) and reproduction factor (Rf). Rf was calculated according to the equation Rf= Pf/ Pi as the Pi (initial population) (Norton, 1978). Egg masses were assessed as described by Daykin and Hussey (1985). Nematode females were collected as described by Mahdy (2002). The galling index was assessed on a scale of 0-10 according to Bridge and Page (1980).

Plant parameters.

Growth characters viz., shoot and root lengths and fresh weights were estimated after 60 days of nematode inoculation. Morever, membrane leakage (ML) was determined as described by Sun *et al*. (2006). The activity of antioxidant enzymes i.e., peroxidase and Polyphenol oxidase (PPO) were measured according to the method described by Fehrman and Dimond (1967) and Broesh (1954), respectively.

RESULTS

Laboratory Experiments:

Effect of homogenized liquid of bacterial and fungal isolates on hatchability of *Meloidogyne* spp. eggs

Percentage of egg hatching inhibition was studied *in vitro* at 28°C by four different bacterial and fungal isolates at different incubation periods *i.e.,* 1, 2, 3 and 7 days. All bioagents exhibited harmful effects on egg hatching

compared to the control. The highest % of hatching inhibition recorded with *B. megaterium* at all different incubation periods, followed by *T. harzianum, P. fluorescens*, *A. brasilense* and *P. polymexa*, respectively compared to control. The highest % of egg hatching inhibition achieved after 7 days with all treatments. The lowest % of hatching recorded after 7 days were 17, 26, 32, 42 and 48%, respectively compared to control as recorded 74% (Table 1).

Table (1): Egg hatching as affected by four liquid bacterial and *T. harzianum* isolates at different incubation periods.

Treatments	% of egg hatching after			
	1 Day	2 Days	3 Days	7 Days
T. harzianum	6	9	18	26
P. fluorescens	11	20	25	32
B. megaterium	3	8	13	17
P. polymexa	18	29	39	48
A. brasilense	14	26	32	42
Control	26	38	54	74

Effect of homogenized liquid of bacterial and fungal isolates on juveniles mortality of *Meloidogyne* spp.

The percentage of juveniles mortality was studied *in vitro* at 28°C by using four liquid bacterial strains *i.e., P. fluorescens*, *B. megaterium*, *P. polymexa* and *A. brasilense* as well as fungus isolate *T. harzianum* at different incubation times *i.e.,* 1, 2, 3 and 7 days. Results revealed that all treatments exhibited nematicidal effects against juveniles compared to control. *B. megaterium* recorded the highest percentage of juvenile's mortality at all intervals times, followed by *T. harzianum, P. fluorescens, A. brasilense* and *P. polymexa*. The highest % of mortality recorded after 7 days of all treatments. The highest mortality % recorded with *B. megaterium* was 84% after 7 days, followed by *T. harzianum* (68%), *P. fluorescence* (38%) and *A. brasilense* (34%), while *P. polymexa* recorded 30% compared to control (14%) (Table 2).

Greenhouse Experiment

Effect of different bioagents at different application times against *Meloidogyne* spp. on eggplant.

The effect of bioagents (*B. megaterium*, *P. fluorescens*, *A. brasilense*, *P. polymexa* and *T. harzianum*) at different application times (before one week, simultaneously and after one week of nematode inoculation) against *Meloidogyne* spp. was investigated. Results cleared that all treatments had the potential to decrease all nematode parameters i.e. galling index, galls, egg masses, female's numbers/root system, J_2/250 g soil as well as nematode reproduction to a large extent compared to plants treated with nematode only. *B. megaterium* showed high reduction in galling index when applied one week prior to nematode inoculation as

recorded 69.2%, (Table, 3). Generally, *P. polymexa* was the less effective one in reducing galling index at all application times.

The reduction % in nematode characters in soil and roots ranged between 47 to 84.9% (Table, 4). Results confirmed that all evaluated biocontrol agents significantly reduced all nematode characters *i.e.,* galls, egg masses, females numbers /root system and J_2/250 g soil. The highest reduction achieved with *B. megaterium,* by 82.9, 84.9, 84.3 and 82%, respectively, especially when inoculated one week prior to nematode inoculation.

Table (2): Juveniles mortality as affected by four liquid bacterial and *T. harzianum* isolates at different incubation periods.

Treatments	% of juvenile's mortality after			
	1 Day	2 Days	3 Days	7 Days
T. harzianum	6	16	33	68
P. fluorescens	4	12	24	38
B. megaterium	8	22	41	84
P. polymexa	2	4	16	30
A. brasilense	3	8	20	34
Control	0	2	8	14

Table (3): Effect of different bioagents at three application times against *Meloidogyne* spp. on eggplant plants.

Treatments	Application times	Galling index	% Reduction
B. megaterium	Before one week	2.7^{ij}*	69.2
	Simultaneously	3.3^{hi}	61.5
	After one week	3.7^{gh}	57.7
P. fluorescens	Before one week	5.3^{de}	38.5
	Simultaneously	5.7^{cd}	34.6
	After one week	6.0^{cd}	30.8
A. brasilense	Before one week	5.7^{cd}	34.6
	Simultaneously	6.7^{bc}	23.1
	After one week	7.0^{b}	19.2
P. polymexa	Before one week	6.67^{bc}	23.1
	Simultaneously	7.3^{b}	15.4
	After one week	7.7^{b}	11.5
T. harzianum	Before one week	3.7^{gh}	57.7
	Simultaneously	4.3^{fg}	50.0
	After one week	4.7^{ef}	46.2
Control		8.7^{a}	

*Columns with different litters are significantly different according to Duncan's Multiple Test (P≤0.05).

In the contrary the lowest effect recorded with *P. polymexa* as the reduction reached 47, 52, 50.9 and 53.3%, respectively when inoculated after one week of nematode inoculation compared to plants treated with nematode alone.

Results showed that all biocontrol agents significantly increased all plant growth parameters i.e., stem and root lengths, fresh shoot and root weights (Table, 5) and the highest efficacy was achieved by *B. megaterium.* In the contrary the lowest efficacy with eggplant plants treated with *P. polymexa* one week after nematode inoculation compared to plants treated with nematode alone.

Table (4): Effect of different bioagents at three different application times, on the management of *Meloidogyne* spp. on eggplant plants under greenhouse conditions.

Treatments	Application times	Nematode parameters/root system						J_2/250 g soil	% Reduction	Pf	Rf *
		No. of galls	% Reduction	Egg masses	% Reduction	Females	% Reduction				
B.	Before one week	93.3^{k}*	82.9	48.7^{j}	84.9	71.0^{j}	84.3	141.7^{l}	82.0	354.7^{m}	0.118^{m}
	Simultaneously	115.0^{jk}	78.9	61.7^{hi}	80.9	79.0^{j}	82.6	163.3^{k}	79.3	419.0^{l}	0.140^{l}
	After one week	122.3^{jk}	77.6	60.7^{i}	81.2	79.0^{j}	82.6	169.3^{k}	78.5	431.3^{kl}	0.144^{kl}
P. fluorescens	Before one week	183.3^{fgh}	66.4	94.3^{f}	70.7	142.0^{h}	68.7	238.3^{i}	69.8	658.0^{h}	0.219^{h}
	Simultaneously	197.7^{fg}	63.7	104.3^{e}	67.6	161.0^{g}	64.5	267.7^{h}	66.0	730.7^{g}	0.244^{g}
	After one week	208.3^{ef}	61.8	113.3^{de}	64.8	167.3^{fg}	63.1	288.3^{g}	63.4	777.3^{f}	0.259^{f}
A. brasilense	Before one week	203.3^{ef}	62.7	115.7^{d}	64.1	175.3^{efg}	61.3	349.3^{cd}	55.7	843.7^{e}	0.281^{e}
	Simultaneously	230.0^{de}	57.8	113.3^{de}	64.8	181.7^{ef}	59.9	308.3^{f}	60.9	833.3^{e}	0.278^{e}
	After one week	253.7^{cd}	53.5	120.7^{d}	62.6	191.00^{de}	57.9	325.0^{e}	58.8	890.3^{d}	0.297^{d}
P. polymexa	Before one week	231.0^{de}	57.6	131.0^{c}	59.4	201.0^{cd}	55.7	338.0^{d}	57.1	901.0^{d}	0.300^{d}
	Simultaneously	275.0^{bc}	49.5	140.0^{c}	56.6	211.0^{bc}	53.5	354.3^{c}	55.1	980.3^{c}	0.327^{c}
	After one week	289.0^{b}	47.0	154.7^{b}	52.0	222.7^{b}	50.9	368.3^{b}	53.3	1034.7^{b}	0.345^{b}
T. harzianum	Before one week	135.0^{ij}	75.2	65.3^{hi}	79.7	96.7^{i}	78.7	175.0^{k}	77.8	472.0^{k}	0.157^{k}
	Simultaneously	158.3^{hi}	71.0	71.7^{h}	77.8	107.3^{i}	76.3	191.7^{j}	75.7	529.0^{j}	0.176^{j}
	After one week	168.3^{gh}	69.1	81.7^{g}	74.7	130.0^{h}	71.3	198.3^{j}	74.8	578.3^{i}	0.193^{i}
Control		545.0^{a}		322.3^{a}		453.3^{a}		788.3^{a}		2109.0^{a}	0.703^{a}

*Columns by different litters are significantly different according to Duncan's Multiple Test (P≤0.05).

Reproduction factor (Rf)= Pf/Pi. Pf= Final population Pi= Initial population

Table (5): Effect of different bioagents at three different application times, on growth characteristics of eggplant plants infected with *Meloidogyne* spp. under greenhouse conditions.

Treatments	Application times	Stem length (cm)	Root length (cm)	Fresh shoot weight (g)	Fresh root weight (g)
B. megaterium	Before one week	36.00^{ab}*	28.67^{ab}	47.15^{ab}	44.20^{ab}
	Simultaneously	35.00^{abc}	27.00^{abc}	42.71^{bc}	41.72^{abc}
	After one week	32.67^{abcde}	22.67^{bcd}	38.62^{cdef}	36.60^{bcde}
P. fluorescens	Before one week	31.00^{bcdef}	23.33^{bcd}	38.03^{cdef}	35.98^{bcde}
	Simultaneously	28.67^{defgh}	22.67^{bcd}	36.24^{def}	33.20^{cdef}
	After one week	26.33^{fgh}	21.67^{bcde}	34.94^{ef}	29.09^{efg}
A. brasilense	Before one week	30.33^{bcdefg}	21.33^{cde}	35.71^{def}	32.57^{cdef}
	Simultaneously	28.33^{efgh}	18.67^{def}	33.41^{fg}	28.58^{efg}
	After one week	25.00^{ghi}	15.33^{ef}	28.84^{gh}	25.97fg
P. polymexa	Before one week	23.67^{hi}	19.00^{def}	27.70^{gh}	30.40^{def}
	Simultaneously	20.33^{ij}	15.00^{ef}	24.60^{h}	27.17^{efg}
	After one week	15.67^{jk}	13.33^{fg}	23.40^{h}	20.67^{gh}
T. harzianum	Before one week	34.33^{abcd}	25.67^{abcd}	40.13^{cde}	36.53^{bcde}
	Simultaneously	29.33^{defgh}	24.00^{bcd}	38.13^{cdef}	37.23^{bcde}
	After one week	28.33^{efgh}	22.67^{bcd}	36.80^{cdef}	33.47^{cdef}
Control		11.00^{k}	8.33^{g}	17.17^{i}	12.75^{h}

*Columns by different litters are significantly different according to Duncan's Multiple Test (P≤0.05).

Determination of some antioxidant enzymes activity and membrane leakage of nematode-infected eggplant treated with different bioagents at three different application times.

Data showed that the highly significant increase in peroxidase and polyphenoloxidase activities obtained with *B. megaterium* when applied before nematode inoculation. In contrary the lowest effect was recorded with *P. polymexa* when applied after nematode inoculation.

Data presented in Table (6) noticed that plants-infected nematodes have a higher membrane leakage (integrity) compared with healthy plants. the higher reduction in integrity recorded with *B. megaterium* treatment. adding before nematode inoculation. In addition, *P. polymexa* gave the lowest reduction in integrity.

Table (6): Effect of different bioagents at three different application times on physiological and antioxidant enzymes of eggplant plants infected with *Meloidogyne* spp.

Treatments	Application times	Peroxidase (O.D.g^{-1} fr.wt.after 2min)	Polyphenoloxidase (O.D.g^{-1} fr.wt. after 45min)	Membrane leakage (%)	Reduction %
B. megaterium	Before one week	1.20^{b}*	1.35^{b}	14.65^{j}	79.86
	Simultaneously	1.17^{b}	1.3^{c}	17.12^{ij}	76.45
	After one week	1.12^{c}	1.28^{c}	18.39^{hij}	74.71
P. fluorescens	Before one week	0.86^{ef}	1.17^{de}	24.8^{fg}	65.90
	Simultaneously	0.83^{fg}	1.13^{ef}	27.98^{f}	61.52
	After one week	0.8^{gh}	1.11^{f}	34.5^{e}	52.56
A. brasilense	Before one week	0.76^{hi}	0.99^{g}	36.08^{de}	50.39
	Simultaneously	0.72^{ij}	0.96^{gh}	37.15^{de}	48.91
	After one week	0.68^{j}	0.94^{gh}	40.65^{d}	44.11
P. polymexa	Before one week	0.59^{k}	0.91^{hi}	49.94^{c}	31.33
	Simultaneously	0.53^{l}	0.87^{i}	53.89^{bc}	25.89
	After one week	0.47^{m}	0.81^{j}	56.59^{b}	22.18
T. harzianum	Before one week	0.93^{d}	1.2^{d}	21.83^{ghi}	69.99
	Simultaneously	0.89^{de}	1.19^{d}	22.37^{fghi}	69.24
	After one week	0.87^{ef}	1.18^{de}	24.25^{fgh}	66.65
Control		0.25^{n}	0.33^{k}	72.72^{a}	

*Columns by different litters are significantly different according to Duncan's Multiple Test (P≤0.05).

DISCUSSION

Bacteria could regulate the development of ecto- and endoparasitic nematodes by different modes of action *in vitro* and *in vivo*. The efficacy of bacterial treatment is better than the effect of chemical pesticides or the same effect.

Moreover, the bacterial application produces additional positive effects on growth stimulation, increases yields and suppresses other pathogenic microorganisms Migunova and Sasanelli (2021). Aballay *et al*., (2013) reported that the culture filtrates resulted from 16 bacterial strains specially the two isolates of *Bacillus megaterium viz;* 69 & 185 were effective against *Meloidogyne ethiopica*. All filtrates decreased egg hatching after 24 h of immersion by 14.3 to 57.1% compared with tryptic soy broth control under laboratory conditions.

The inhibition of egg hatching was attributed to the secondary metabolites produced by the rhizobacteria, which caused egg lysis and affected egg viability. *Bacillus megaterium* plays an important role in dissolving the

unavailable phosphorus compounds in soil rendering them available for growing crops. Peroxidase and polyphenol oxidase is thought to reinforce cell walls (lignification and suberization) at the border of infection and further limit the spread of pathogens (Mostafa *et al*., 2018). A plant growth-promoting rhizobacterium, *Bacillus megaterium* YMF3.25, was demonstrated to be an efficient biocontrol agent against root-knot nematode *Meloidogyne incognita*.

Results from laboratory tests and a greenhouse experiment indicated that the bacterial culture could significantly inhibit egg hatching and reduce infection of the nematode through the production of nematicidal volatiles.

After analysis by gas chromato-graphy/mass spectrometer and confirmation with commercial pure compounds, the nematicidal volatiles produced by the bacterium was characterized to include mainly the benzene acetaldehyde, 2-nonanone, decanal, 2-undecanone and dimethyl disulphide, which were active against both juveniles and eggs at the concentration of 0.5 m mol.

Six compounds (phenyl ethanone, nonane, phenol, 3,5-dimethoxy-toluene, 2,3-dimethyl- butanedinitrile and 1-ethenyl-4-methoxy- benzene) with nematicidal activities of 30–63% also contributed to nematicidal efficacy of the bacterium. *Bacillus megaterium* promoted growth and altered root-system architecture through an auxin- and ethylene-independent signaling mechanism in Arabidopsis thaliana.

The potential of *Bacillus megaterium* as a biological control agent (BCA) against plant parasitic nematodes on *Meloidogyne chitwoodi*. Crude metabolites produced by *B. megaterium* caused a significant reduction in the number of root galls and eggs as mentioned by Huang *et al*., (2010). *Bacillus megaterium* is involved in chitin degradation, nitrogen fixation, or solubilization of insoluble phosphates.

An endophytic isolate of *B. megaterium*, which was found in root nodules of *Medicago polymorpha*, was able to produce indole acetic acid (IAA) as reported by Rostami *et al*., (2021). *Bacillus megaterium* shows enhanced incorporation of organic matter that activates antibiosis towards the nematode activity and enhances crop production as reported by Osman *et al*., (2021). *Bacillus megaterium* isolate proved to be the most efficient biofertilizer and nematicidal as reported by El-Hadad *et al*., (2010). *Bacillus megaterium* DS9 demonstrated active antinematode either *in vitro* or in the greenhouse tests and also had a good effect on plant growth parameters Tran *et al*., (2019).

Mahdy *et al.,* (2000) confirmed that all crops treated with *B. cereus* S18 combined with *M. incognita* showed plant growth improvement when compared with the bacteria untreated crops. Suppressing nematode damage with rhizobacterial strains improved tomato root weight, which might also account for some of the detected suppression; such as reducing galls, and

stopping renewal root tips. This may stop their growth or cause extreme branching of roots, paving the way to the normal function of roots such as the uptake and transport of water and nutrients. Encouraging impact extended to improve plant biomass and height. These results also agreed with those obtained by (Padgham and Sikora 2007; Saikia *et al.,* 2013; Youssef *et al*., 2017 and Engelbrecht *et al*., 2018).

Author Contributions:

Conceptualization, EMM, develop the research data, NMG, MEM and MHS implementing and conducting the researchm, record the results, data curation, formal analysis, investigations, methodology, EMM, MEM, MHS and NMG writing original drafts, and writing and editing; all authors have read and agreed to the purplish version of the manuscript.

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