Research Article

Integrated Effect of Plant Growth Promoting Rhizobacteria with *Trichoderma Viride* on Root Knot Nematode Infected Eggplant

Marwa M. El-Deriny[1,2*], Rania H. Wahdan[1], Marwa S. Fouad[3] and Dina S.S. Ibrahim[1,2*]

[1]Nematode Diseases Research Department, Plant Pathology Research Institute., Agricultural Research Center (ARC), Giza, Egypt; [2]The Egyptian Nematology Reference Lab (ENR lab) and Genetic Diversity of Microbial Plant Pathogens Reference Lab, Plant Pathology Research Institute., Agricultural Research Center (ARC), Giza, Egypt; [3]Seed Pathology Research Dept., Plant Pathology Research Institute., Agricultural Research Center (ARC), Giza, Egypt.

Abstract | *Meloidogyne* spp. can infect various horticultural plants, including eggplants (*Solanum melongena*, L) and cause destructive loss in crop yield production. The purpose of this study was to evaluate the impact of individual or concomitant treatments with *Trichoderma viride* and some plant growth promoting rhizobacteria (PGPR) on root knot nematode in eggplant.In greenhouse, a standard microbial fertilizer (NPK) and four microbial isolates involved *Pseudomonas fluorescens*, *Serratia marcescens*, *Bacillus subtilis*, and *Trichoderma viride* were tested and it was found thatthe dual treatments composed of NPK and *Pseudomonas fluorescens* or *T. viride,* significantly reduced the total population of root-knot nematode, *M. javanica*, with a reduction percentage of 67.23 and 71.02% respectively. Also, concomitant treatment of NPK and *T. viride* or *B. subtilis* improved the plant growth parameters with the 70.82 and 48.60% respectively. Under field conditions, the growth parameters increased significantly as shoot length (59.98%), shoot weight (114.06%), shoot dry weight (163.97%) and yield per plant (153.06%) upon the treatment with *T. viride* incorporated with NPK compared to the untreated control under the infection stress. Moreover, the concomitant treatment of NPK and *T. viride* gave the highest reduction percentage in the total nematode population (52.5%) followed by treatment with NPK associated with *P. fluorescens* (42.93%). Furthermore, a highest negative correlation was found between the plant yield and the final nematode population in soil and root. Meanwhile, total chlorophyll content increased significantly recorded the highest value upon the application of NPK integrated with *T. viride* under the infection stress while carotenoids content decreased compared to the untreated plants.On the other hand, the use of *T. viride* with NPK or *S. marcescens* significantly increase the content of carbohydrates in the plant leaves. Application of NPK with *P. fluorescens* significantly decreased the proline content and malondialdehyde (MDA). Concomitant treatments using NPK integrated with *T. viride* caused obvious increase in nitrogen (N), phosphorous(P) and potassium (K) concentrations in leaves. It was concluded that the motivating efficiency of PGPR and *Trichoderma* in suppressing plant parasitic nematodes and improving growth yield, encourages the future researches to highpoint the fungal and bacterial interactions with plants as biological control agents for ecological remediation.

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***Correspondence** | Marwa M. El-Deriny and Dina S.S. Ibrahim, Nematode Diseases Research Department, Plant Pathology Research Institute., Agricultural Research Center (ARC), Giza, Egypt; **Email:** marwaelderiny@gmail.com and Dina.Serag@arc.sci.eg.
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Introduction

The recent studies have reported the presence of about 4100 species of plant-parasitic nematodes worldwide that caused asignificant negative influence on the economic cropsestimated at 12.3% of global yield loss ($157 billion) (Singh *et al*., 2015). The damage resulted from nematode infection was greater than that of the invading insects, which had been estimated to being around US$70 billion (Bradshaw *et al*., 2016). Since the symptoms of nematode infection in plants were frequently non-specific, made it difficult to link crop losses to nematode damage, the full extent of nematode impairment globally had been likely to be underestimated (Jones *et al*., 2013; Siddique and Grundler, 2018). Food quality and visual flaws linked to infection symptoms may result in further losses (Palomares-Rius *et al*., 2017). One of the main obstacles to eggplant production is the root-knot nematode (Zeerak *et al*., 2017). According to Anwar and McKenry (2010), the plants infected with nematodes have a smaller root system with fewer feeder roots.

Owing to the significant economic damage that parasitic nematodes can inflict, numerous nematode-control strategies have been developed for the agricultural sector. The use of alive organisms to reduce a pest organism's population density or impact as well as render the pest injury have been known as biological control. Poveda *et al*. (2020) mentioned that biological control of nematodes is specifically defined as the management of nematode populations and/or a decrease nematode damage by organisms that are antagonistic to them. These antagonists can be introduced, naturally occurring, or result from environmental manipulation. Many plant species have been shown to benefit from the application of bacteria and fungi in their rhizosphere, which can both protect the plant against disease and insect attacks and promote plant development. In accordance with Bhattacharyya and Jha (2012), rhizobacteria that stimulate plant growth are important biological resources. Liu *et al*. (2016) refer to that they can boost agricultural yields and strengthen plants' defenses against harmful pests.

Conferring to Ibrahim *et al*. (2020), fungi such as *Trichoderma* were frequently isolated from soils, were not only saprophytic in nature but also served as the egg parasites of plant-parasitic nematodes. These fungi, however, were simple to grow in large quantities and had little trouble colonizing the root surface. Moreover, these fungi function as a bionematicide against nematodes and interfered with other microbes' space and nutrition (Fan *et al*., 2020). Fascinatingly, plant growth-promoting rhizobacteria (PGPRs) had the ability to colonize plant roots and promoted plant growth. Meanwhile, certain PGPRs had also been shown to exhibit antimicrobial activity against plant-parasitic nematodes (Aioub *et al*., 2022). The secondary metabolites produced by *Pseudomonas fluorescens* cause the death of worm eggs and second-stage infectious juveniles (Siddiqui and Shaukat, 2003). In a studyperformed by Zhao *et al*. (2018), five bacterial strains: *Bacillus subtilis*, *B. cereus*, *Serratia proteamaculans*, *Pseudomonas fluorescens* and *P. putida* showed great efficiency as biological control agents against *Meloidogyne javanica* out of 860 strains that were collected from the rhizosphere. Therefore, PGPRs have enormous potential through direct interaction against plant pathogens like nematodesin addition to being beneficial for plant growth (Backer *et al*., 2018).

As bioagents and biofertilizers are easier to apply and less expensive than inorganic fertilizers, farmers have recently become more interested in using them (Babalola *et al*., 2021; Akanmu *et al*., 2023). Thus, the purpose of this study was to estimate the effectiveness of certain microbial bioagents, namely:

NPK(the standard microbial fertilizer),*P. fluorescens*, *S. marcescens*, *B. subtilis*, and *T. viride*, either alone or in combination, for management *M. javanica*as well as enhancing eggplant growth and yield production in both greenhouse and openfield.

Materials and Methods

Pure Nematode Culture

In accordance with Hussey and Barker's (1973) method, nematode (*M. javanica*) eggs were extracted from diseased coleus roots (*Coleus blumei* L.) using a sodium hypochlorite solution. Every day, second-stage nematode juveniles (J2s) were removed from the eggs and stored at 15 °C. The juveniles used in the tests were only five days old.

Preparation of biocontrol agents

NPK is a common microbial fertilizer that contains three different microorganisms: *Bacillus megaterium*, *B. circulans*, and *Azotobacter chroococcum* (1:1:1). *Pseudomonas fluorescens*, *Serratia marcescens*, *B. subtilis*, and *Trichoderma viride* were the other four microbial isolates that were supplied by the Central Lab of Organic Agriculture at the Agricultural Research Centre. According to Abdellatif *et al.* (2021), the various bacterial treatments were prepared as liquid cultures grown on modified nutrient broth (NB) medium containing 10^8cfu/ml, while *T. viride* was performed as a homogenized growth culture suspension containing 10^6cfu/mlusing a technique that was mentioned by Sayed *et al.* (2019).

Nematicide

Oxamyl: (Vydate 10% G.) Methyle – N – N – dimethyl – (N (methyle) carbomycocyl) – 1 - hioxamidate, was applied at the recommended dose.

Greenhouse experiment

Twenty-five-day-old egg plant *cv.* Black Roumy seedlings were transplanted one at a time into 15 cm-d plastic pots that were filled with 850 g of steam-sterilized sandy-loamy soil. When the seedlings were transplanted, they received a 1000 J_2 inoculation of *M. javanica*. They also received individual and combined treatmentsat a rate of 20 ml/pot with *P. fluorescens*, *S. marcescens*, *B. subtilis*, *T. viride*, and NPK, as a standard microbial fertilizer (Table 1). Two days following nematode inoculation, the standard nematicide oxamyl was applied at the recommended dosage (0.3 g/plant/pot). Pots with nematode inoculum were designated as CK1, while pots without nematode inoculum were designated as CK2. Experiment was arranged as five replicates for each treatment. Water was applied to each plastic pot as needed, and they were all set up in a complete block design system with identical agronomical treatments at a temperature of 26±5°C in a greenhouse.

Plants were harvested 45 days following nematode inoculation. For each treatment, results were recorded regarding the length, fresh weight of shoot and root, and dried weight of the shoot. Using a modified Baermann approach, second stage juveniles of *M. javanica* were recovered from the soil (Goodey, 1957) and counted. Roots were cleaned with tap water and dyed with acid fuschin lactic acid (Byrd *et al.*, 1983) then they were immersed in pure cold glycerin. Using a stereomicroscope, the number of galls, egg masses, females, and developmental phases were counted.

Table 1: *Number of treatments from 1 to 18.*

1-NPK (a standard microbial fertilizer)	10- *B. subtilis* + *P. fluorescens*
2-*Bacillus subtilis*	11- *B. subtilis* + *S. marcescens*
3-*Pseudomonas fluorescens*	12- *B. subtilis* + *T. viride*
4-*Serratia marcescens*	13- *P. fluorescens* + *S. marcescens*
5-*Trichoderma viride*	14- *P. fluorescens* + *T. viride*
6-NPK + *B. subtilis*	15- *S. marcescens* + *T. viride*
7-NPK + *P. fluorescens*	16- Oxamyl
8-NPK + *S. marcescens*	17- Nematode alone (CK1) (positive control)
9-NPK + *T. viride*	18- Healthy plant (CK2) (negative control)

Field experiment

In Wadi Elnatron, El-Behira governorate, a micro-plot field experiment was set up to evaluate the nematicidal properties of specific bioagents against root-knot nematode-infected eggplant (*Solanum melongena*, L.) cv. Black roumy. The root-knot nematode *Meloidogyne* spp. (590 juveniles / 250 g soil) naturally colonized the plots. A field experiment was designed as a randomized complete block design (RCBD) and four replications occupied a total area of 175 m2. Eight treated plots and an untreated control were included in each block. A plot was 60 centimeters broad by 6 meters long. It had four rows. Next, a single hill of seedless eggplant variety, cv. Black Roumy, was planted in each plot.

Treatments listed in Table 2 were supplied twice at

a concentration of 100 milliliters per plant as soil drenching, the first was after seven days of planting and the second was after one month later. Additionally, 1g of oxamyl was administered each plant.

Table 2: *Listed treatments in open field.*

1- NPK + *B. subtilis*	6- *T. viride* + *P. fluorescens*
2- NPK + *P. fluorescens*	7 - *T. viride* + *S. marcescens*
3- NPK + *S. marcescens*	8- Oxamyl
4- NPK + *T. viride*	9 - Nematode alone
5- *T. viride* + *B. subtilis*	

Plants were collected after two months of planting and the roots were cleaned of any remaining soil sticking to them. Records were kept on the weight of fruits, shoot dry weight, shoot and root lengths, and fresh shoot and root weights. A composite soil (250g) was prepared from each plot using the modified Baermman technique and sieving in order to extract nematodes (Goodey, 1957). Byrd *et al.* (1983) stated that staining was done on one gram of each plant. The number of egg masses and galls was counted using a stereomicroscope.

Biochemical markers of resistance in eggplants

For measurement of the concentration of Nitrogen (N), phosphorous(P) and potassium (K) concentrations in leave, leaf samples were finely dried in an oven set to 70 °C, and then wet digested as demonstrated by Mertens (2005a; b) and Agrilasa (2002). Photosynthetic pigments were determined in accordance with (Vernon and Seely 2014; Licthenthaler, 1987). To assess the pigment content, fresh 0.5 g leaf tissue was ground with a crusher and mortar in 80% acetone and the centrifuged for five minutes at 10,000 ×g. Carotenoid, chlorophyll a, and chlorophyll b concentrations were measured spectrophotometrically as the absorbance at 470, 652, and 665 nm respectively.

The Umbreit *et al.* (1964) method was reported to evaluate the carbohydrate content in the dried tissues of eggplants. Briefly, the dried shoots (0.5 g) were ground in 5 ml of 30% trichloroacetic acid (TCA) and 2.5 ml of 2% phenol then filtered through filter paper.1 ml of the filtrate was treated with 2 ml of anthrone reagent (2 g anthrone/L of 95% H_2SO_4) and the absorbance of the created blue-green color was measured at 620 nm.

However, the total phenolics were assayed using the tried-and-true methodology of Dai *et al.* (1993). For at least 24 hours, one gram of plant tissue was extracted in 5–10 ml of 80% ethanol. The residue was twice extracted using the same solvent following filtering. Every extract was finished with 50 milliliters of 80% ethanol. After thoroughly combining the extract (0.5 ml) with 0.5 ml of Folin's reagent, the mixture was shaken for three minutes. After adding 3 ml of distilled water to a 1 ml saturated Na_2CO_3 solution, everything was thoroughly homogenized. A spectrophotometer was used to measure the blue color after an hour at a wavelength of 725 nm.

The soluble protein content was determined as mentioned by Lowry *et al.* (1951). In short, 0.5 ml of Folin's reagent (diluted 1:3 v/v) and 1 mL of plant extract were mixed with 5 mL of alkaline reagent (50 ml of 2% Na_2CO_3 prepared in 0.1 N NaOH and 1 ml of 0.5% $CuSO_4$ prepared in 1% potassium sodium tartrate). A shift in color was visible at 750 nm after 30 minutes. Hu *et al.* (2004) investigated the contents of malondialdehyde (MDA). The molar coefficient of absorbance of 155 mmol L^{-1} cm^{-1} was used to calculate the MDA concentration, which was then reported as $nmolg^{-1}$ FW. In addition, the activities of catalase and superoxide dismutase were measured using techniques outlined by Bergmeyer (1974).

The proline content was assessed free of charge using the procedure outlined by Bates *et al.* (1973). 10 milliliters of 3% sulfosalicylic acid were used to homogenize 0.5 gram of dried plant material. Following filtering, 2 ml of filtrate and 2 ml of glacial acetic acid reacted with 1.25 g of ninhydrin in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid, stirring until dissolved. After an hour in a bath of boiling water, this reaction was placed in an ice bath. Lastly, 4 milliliters of toluene were used to extract the reaction mixture. After being removed from the aqueous phase, the toluene-containing chromophore was measured spectrophotometrically at 520 nm.

Statistical analysis

Data were analyzed using SPSS Statistical analysis for windows version, 26. Tukey–Kramer test for multiple comparisons with $p \leq 0.05$ level of probability as the significance level. Correlation was measured at $p \leq 0.01$.

Results and Discussion

Greenhouse experiment

The obtained data in Table 3 demonstrated that all

Table 3: *The effects of specific microbial bioagents, either alone or in combination, on the plant growth characteristics of eggplant (Solanum melongena, L.) infected with Meloidogyne javanicain greenhouse.*

Treatment	shoot L.	root L.	shoot wt.	root wt.	fresh wt.	Increase %	dry wt.
NPK	24.46^{def}	15.52^{d}	18.91^{defg}	2.60^{ghi}	21.51^{defg}	22.84	1.91^{ghi}
B	18.98^{hi}	15.00^{de}	19.20^{defg}	2.23^{ijk}	21.43^{defg}	22.38	2.11^{efg}
P	26.52^{cde}	11.02^{f}	17.21^{g}	2.11^{jkl}	19.32^{gh}	10.33	1.70^{i}
S	29.00^{bc}	11.02^{f}	20.32^{bcdefg}	1.91^{kl}	22.23^{cdefg}	26.96	2.40^{cdef}
T	30.10^{bc}	16.02^{cd}	23.14^{ab}	2.50^{ghij}	25.64^{b}	46.43	3.11^{b}
NPK+B	30.10^{bc}	18.98^{b}	22.52^{bc}	3.50^{bcd}	26.02^{b}	48.60	3.22^{b}
NPK+P	32.50^{ab}	12.02^{f}	20.91^{bcdef}	3.40^{bcd}	24.31^{bcd}	38.83	3.11^{b}
NPK+S	28.06^{cd}	18.02^{bc}	19.62^{cdefg}	2.89^{fgh}	22.51^{cdefg}	28.56	2.60^{cd}
NPK+T	35.48^{a}	22.02^{a}	25.61^{a}	4.30^{a}	29.91^{a}	70.82	3.92^{a}
B + P	22.02^{fgh}	13.04^{ef}	17.99^{fg}	2.70^{ghi}	20.69^{efg}	18.16	1.80^{hi}
B+S	29.02^{bc}	17.04^{bcd}	21.31^{bcde}	1.80^{l}	23.21^{bcdef}	31.98	2.50^{cde}
B + T	27.00^{cd}	16.52^{cd}	18.61^{efg}	3.79^{b}	22.40^{cdefg}	27.93	1.70^{i}
P + S	20.02^{ghi}	15.00^{de}	19.91^{cdefg}	3.61^{bc}	23.52^{bcde}	34.32	2.70^{c}
P+T	24.98^{def}	12.02^{f}	21.82^{bcd}	3.10^{efg}	24.92^{bc}	42.32	2.30^{def}
S + T	22.02^{fgh}	13.04^{ef}	19.62^{cdefg}	2.70^{ghi}	22.32^{cdefg}	27.47	2.23^{def}
Oxamyl	20.02^{ghi}	15.00^{de}	17.50^{g}	2.41^{hij}	19.91^{fgh}	13.71	1.80^{hi}
Nematode alone (CK1)	17.52^{i}	8.02^{g}	14.30 h	3.21^{def}	17.51^{h}	--	1.60^{i}
Healthy plant (CK2)	23.00^{efg}	13.04^{ef}	18.91^{defg}	2.80^{fghi}	21.71^{defg}	23.99	2.11^{efg}

** Within each column, a different letter(s) indicates significantly different (Tukey test at probability ≤ 0.05).*
NPK: *a standard microbial fertilizer;* **B:** *Bacillus subtilis;* **P:** *Pseudomonas fluorescens;* **S:** *Serratia marcescens;* **T:** *Trichoderma viride.*

tested treatments clearly much improved the growth characteristics of eggplant plants. Nevertheless, concurrent therapies produced superior outcomes than solitary ones. It was obvious that concomitant treatments of NPK and *T. viride* or *B. subtilis* gave the highest results in terms of enhancing plant length, shoot dry weight, and total plant fresh weight, with percentages of 70.82 and 48.60% in total plant fresh weight, respectively. But the treatment with *T. viride* alone improved plant growth parameters with the increase percentage of (46.43%). The impact of other isolates on plant growth criteria varied considerably.

The entire nematode population was considerably decreased upon the application of the all-tested treatments as shown in Table 4. With the same manner to growth parameters measurements,the concomitant treatments produced superior consequences than the individual ones. The most effective dual actions among those that were examined were NPK with either *T. viride* or *P. flurescens*, which considerably reduced the overall population of root-knot nematodes by 71.02 and 67.23% respectively. Nonetheless, *S. marcescens* showed the least amount of suppression in the nematode population in soilbeing (19.18%). The best standard nematicide was oxamyl, which also demonstrated the greatest reduction in egg masses and root galling, as well as the highest reduction in the total nematode population (92.44%).

Additionally, the plant growth parameters mainly plant fresh and dry weight were significantly affected with the change in the three distinct traits: egg masses, galls, and total number of nematodes in soil and roots with a strong correlation as shown in Table 5. Consequently, the nematodes had a detrimental effect on the plants. The plant's fresh and dry weight decreases with an increase in *M. javanica* population.

Field experiment

Through the previous greenhouse investigation, the most effective treatments for reducing thenematode population and increasing the vegetative growth parameters measurements to be studied in the field were attributed to NPK (a conventional microbial fertilizer) or the fungus *T. viride* concurrent with the other three microbial isolates.The impact of fungus *T. viride* combined with NPK treatment resulted in a remarkable

Table 4: *Nematode population in soil, Number of root galls and egg masses of Meloidogyne javanica infecting eggplant (Solanum melongena, L.) cv. Black roomy as influenced by certain microbial bioagents in comparison with oxamyl in greenhouse.*

Treatment	Nematode	Develop	Females	Total	Reduction %	Galls	Egg masses
NPK	2000 ^{e}	25.00^{efg}	78.0 ^{de}	2103 ^{f}	41.79	92 ^{de}	60 ^{cd}
B	2572^{bc}	45.00 ^{b}	110.0 ^{b}	2727^{bcd}	24.52	120 ^{b}	78 ^{b}
P	2700^{bc}	27.00^{efg}	96.0 ^{c}	2823^{bc}	21.86	100 ^{c}	81 ^{b}
S	2788 ^{b}	39.00 ^{c}	93.0 ^{c}	2920 ^{b}	19.18	119 ^{b}	78 ^{b}
T	2630^{bc}	20.00 ^{h}	50.0^{gh}	2700^{bcd}	25.27	53 ^{i}	35 ^{g}
NPK+B	1780 ^{e}	20.60^{gh}	69.0^{ef}	1869.6 ^{f}	48.27	75^{fg}	57 ^{de}
NPK+P	1090 ^{f}	31.40 ^{d}	63.0 ^{f}	1184.4 ^{g}	67.23	86^{ef}	50^{ef}
NPK+S	1800 ^{e}	23.60^{fgh}	53.0 ^{h}	1876.6 ^{f}	48.07	65^{gh}	46 ^{f}
NPK+T	990 ^{f}	15.00 ^{i}	42.0 ^{h}	1047 ^{g}	71.02	44 ^{i}	33 ^{g}
B + P	2368 ^{cd}	31.00 ^{d}	72.0^{ef}	2471 ^{de}	31.61	89 ^{de}	60 ^{cd}
B+S	2088 ^{de}	41.00^{bc}	77.0 ^{de}	2206 ^{f}	38.94	99 ^{c}	56 ^{de}
B + T	2688^{bc}	28.00^{efg}	79.0 ^{de}	2795^{bcd}	22.64	91 ^{de}	64 ^{cd}
P + S	1900 ^{e}	24.00^{fgh}	83.0 ^{d}	2007 ^{f}	44.45	81^{ef}	69 ^{c}
P+T	2500^{bc}	38.00 ^{c}	41.0 ^{h}	2579^{cd}	28.62	55 ^{i}	28 ^{g}
S + T	1988 ^{e}	29.00 ^{de}	44.0^{gh}	2061 ^{f}	42.96	57 ^{h}	33 ^{g}
oxamyl	260 ^{g}	7.00 ^{j}	6.0 ^{i}	273.04 ^{h}	92.44	10 ^{j}	3 ^{h}
Nematode alone (CK1)	3400 ^{a}	63.00 ^{a}	150.0 ^{a}	3613 ^{a}	--	188 ^{a}	120 ^{a}

Within each column, a different letter(s) indicates significantly different (Tukey test at probability ≤ 0.05).
NPK: *a standard microbial fertilizer,* **B:** *Bacillus subtilis,* **P:** *Pseudomonas luorescens,* **S:** *Serratia marcescens,* **T:** *Trichoderma viride.*

Table 5: *Correlation between the plant fresh and dry weight with the different nematode's parameters.*

Nematode's parameters	Plant fresh weight	Plant dry weight
Total nematode	0.340**	0.408**
Galls	0.456**	0.361**
Egg masses	0.432**	0.337**

**Correlation is significant at p ≤ 0.01.

increase in shoot length (59.98%), shoot weight (114.06%), shoot dry weight (163.97%), and yield per plant (153.06%) as compared to untreated plant. Additionally, the integration of *T. viride* with *S. marcescens* or *P. fluorescens* results in a considerable rise in all eggplant growth parameters measurements.

Table 6: *Plant development metrics of eggplant cultivated in naturally Meloidogyne-infested soil treated with certain microbial bioagents compared to oxamyl under field conditions*

Treatment	shoot L.	Inc.%	shoot wt.	Inc.%	dry wt.	Inc.%	yield/plant (g.)	Inc.%
NPK+B	69.08 cd	15.09	249.05 e	36.69	48.59 c	62.56	330 c	34.69
NPK+P	85.96 b	43.22	301.04 bc	65.23	65.31 b	118.5	450 b	83.67
NPK+S	75.04 c	25.02	289.87 de	59.10	61.19 b	104.72	305 c	24.48
NPK+T	96.02 a	59.98	390.00 a	114.06	78.90 a	163.97	620 a	153.06
T+B	88.06 b	46.72	275.10 cde	50.99	53.33 c	78.42	299 c	22.04
T+P	91.98 ab	53.25	329.96 b	81.11	67.50 b	125.83	402 b	64.08
T+S	89.90 ab	49.78	264.00 de	44.9	51.42 c	72.03	281 cd	14.69
Oxamyl	66.02 de	9.99	251.98 e	38.1	47.89 c	60.22	319 c	30.20
Nematode alone	60.02 e	--	182.19 f	--	29.89 d	--	245 d	--

Within each column, a different letter(s) indicates significantly different (Tukey test at probability ≤ 0.05).
NPK: *a standard microbial fertilizer,* **B:** *Bacillus subtilis,* **P:** *Pseudomonas fluorescens,* **S:** *Serratiamarcescens,* **T:** *Trichoderm aviride*

Table 7: *Influence of certain microbial bioagents on population reduction of Meloidogyne spp. infected eggplant under field conditions.*

Treatment	Finalpopulation	Red. %	galls	Red. %	Eggmasses	Red. %
NPK+B	2388 cd	35.11	120 d	50.00	98 de	43.02
NPK+P	2100 d	42.93	93 e	61.25	72 f	58.14
NPK+S	3148 b	14.46	190 b	20.83	141 b	18.02
NPK+T	1748 e	52.5	88 e	63.33	61 f	64.53
T+B	2620 c	28.80	181 e	24.58	122 c	29.07
T+P	2268 d	38.37	126 cd	47.50	107 d	37.79
T+S	2708 c	26.41	140 c	41.67	90 e	47.67
Oxamyl	1250 f	66.03	52 f	78.33	21 g	87.79
Nematode alone	3680 a	--	240 a	--	172 a	--

Within each column, a different letter(s) indicates significantly different (Tukey test at probability ≤ 0.05).
NPK: a standard microbial fertilizer, **B:** *Bacillus subtilis*, **P:** *Pseudomonas fluorescens*, **S:** *Serratia marcescens*, **T:** *Trichoderma viride.*

Table 7 displayed that all treatments that was applied in field considerably reduced the nematode population in the soil and roots of *S. melongena* under the infection stress. Combined treatments of NPK and the fungus *T. viride* recorded the largest reduction in the total nematode population (52.5%), while concurrent NPK treatment with *P. fluorescens* came to the second-in order (42.93%). The number of galls and egg masses showed a similar pattern, both of which were dramatically reduced by all treatments. The incorporation of *T. viride* and NPK resulted in the greatest percentage reduction in egg masses (64.53%) and root galling (63.33%).

Moreover, it was found that the plant's weight yield was significantly decreased with the increase nematode infection represented by the increase in the three distinct traits: egg masses, galls, and final population of nematodes in soil and roots with a strong correlation (where r = 0.461, 0.531 and 0.538 respectively at P≤ 0.01) as shown in Table (8).

Nitrogen (N), Phosphorus (P) and Potassium (K) constituents

Table 9 demonstrated that potassium (K), phosphorous (P), and nitrogen (N)levels increased in the eggplants upon the treatment with microbial bioagents. The highest value of nitrogen content was obtained with the application of NPK treatments combined with either *B. subtilis* or *T. viride.* Moreover, *T. viride* combined with NPK improved the phosphorous concentration. Regarding potassium content, it was shown that addition of *S. marcescens*, *P. fluorescens*, *T. viride*, or *B. subtilis* to conventional microbial fertilizer NPK considerably improved potassium content, respectively.

Table 8: *Correlation between the weights of yield per plant with the different nematode's parameters.*

Nematode's parameters	yield/plant
Final population of nematode	0.538**
Galls	0.531**
Egg masses	0.461**

***Correlation is significant atp ≤ 0.01.*

Table 9: *Concentrations of nitrogen (N), phosphorous (P), and potassium (K) in eggplant leaves infested with Meloidogyne spp. and treated with specific microbial bioagents.*

Treatment	N (mg/g)	P (mg/g)	K (mg/g)
NPK+B	3.81 b	0.578 c	2.47 b
NPK+P	3.59 bc	0.692 b	3.02 a
NPK+S	2.97 d	0.684 b	3.22 a
NPK+T	4.25 a	0.764 a	2.96 a
T+B	2.84 d	0.548 cd	1.99 cd
T+P	3.25 cd	0.328 e	2.04 cd
T+S	2.78 d	0.494 d	2.35 bc
Oxamyl	2.95 d	0.226 f	2.01 cd
Nematode alone	1.99 e	0.212 f	1.85 d

Within each column, a different letter(s) indicates significantly different (Tukey test at probability ≤ 0.05).
NPK: *a standard microbial fertilizer,* **B:** *Bacillus subtilis,* **P:** *Pseudomonas fluorescens,* **S:** *Serratia marcescens,* **T:** *Trichoderma viride*

Table 10: *Biochemical markers of resistance in eggplants infected with Meloidogyne spp. and responded to specific microbial bioagent treatments.*

Treatment	Chl a	Chl b	Carotenoid	Carbohydrate	Phenol	Protein	MDA	Catalase	Superoxidase	Proline
NPK+B	22.93 b	19.28 b	1.61 ef	75.12 bc	2.33 d	1.92 bc	9.75 ab	0.61 d	1.69 d	7.36 cde
NPK+P	21.43 bc	18.61 b	2.02 d	64.81 d	2.12 de	2.54 a	7.04 e	0.84 b	2.93 ab	5.39 f
NPK+S	23.42 ab	20.56 ab	1.87 de	54.34 e	1.87 e	2.51 a	7.68 de	0.37 e	2.61 bc	7.68 bcd
NPK+T	25.62 a	21.82 a	1.38 f	82.63 a	1.53 f	2.82 a	7.23 de	0.59 d	3.23 a	6.42 e
T+B	19.67 cd	15.95 c	2.41 bc	68.87 cd	2.98 b	2.57 a	9.02 bc	0.65 cd	3.20 a	6.89 de
T+P	22.40 b	19.62 ab	2.15 cd	71.79 bcd	1.98 e	2.08 b	8.11 cde	1.00 a	1.95 d	8.25 abc
T+S	18.71 d	16.24 c	2.57 b	78.73 ab	2.06 de	1.69 cd	7.98 cde	0.74 c	2.76 bc	7.01 de
Oxamyl	16.24 e	13.07 d	2.11 cd	73.82 bc	2.65 c	1.84 bc	8.35 cd	0.98 a	2.53 c	8.45 ab
Nematode alone	10.53 f	9.62 e	3.12 a	41.24 f	3.56 a	1.39 d	10.56 a	1.01 a	1.92 d	9.20 a

Within each column, a different letter(s) indicates significantly different (Tukey test at probability ≤ 0.05).
NPK: *a standard microbial fertilizer,* **B:** *Bacillus subtilis,* **P:** *Pseudomonas fluorescens,* **S:** *Serratia marcescens,* **T:** *Trichoderma viride*

Biochemical markers of resistance in eggplants

The most notable and substantial rise in total chlorophyll was observed upon the application of NPK combined with the fungus *T. viride* compared to the untreated control. However, carotenoids content decreased significantly with the concurrent treatment of NPK and *T. viride* or *B. subtilis*.

The application of *T. viride* combined with NPK or *S. marcescens* treatments greatly raises the plant's leaf carbohydrate content. The various treatments had an impact on eggplants' phenolic compounds concentration that was considerably reduced by the application of *T. viride* treatment combined by NPK. Moreover, the protein content was greatly increased with the dual treatment of NPK and *P. fluorescens, T. viride*, or *S. marcescens* strains. A similar pattern was observed when *B. subtilis* and *T. viride* was used as a therapy. Plants infected with *Meloidogyne* spp. accumulated more MDA than uninfected plants. A noticeable drop in MDA level was found following the application of NPK combined with *P. fluorescens*.

As oxidative enzymes in the plant affected by the infection conditions, it was found that treatment with *T. viride* concomitant with *B. subtilis* or NPK greatly increased the superoxidase activity but the application of NPK treatment combined with *S. marcescens* resulted in a considerable drop-in catalase activity compared to the untreated control Additionally, proline content was greatly reduced when *P. fluorescens* was used in conjunction with NPK (Table 10).

Root-knot nematodes readily target high-value crops in Egypt, including eggplant, resulting in lower yields (Shaaban *et al.*, 2023). Numerous biological resources, such as microbial bioagents that are crucial for plant protection, have been employed to manage root knot nematodes. In developing nations, prolonged usage of fertilizers and pesticides leads to serious ecological issues. *Meloidogyne* spp. can interact with other pathogens and generate complex diseases, making prevention and control of the species in the soil challenging. Thus, screening multifunctional microbial agents is crucial for increasing soil nutrient content and managing *Meloidogyne* spp. as they may control nutrient transformation, nutrient acquisition, utilization, and crop sustainability, rhizosphere microbes are extremely important (Prasad *et al.*, 2017). Through nitrogen fixation, plant hormone generation, mineral solubilization, iron carrier and HCN production, and other processes, rhizosphere microflora promotes plant growth under abiotic stress and activates defense mechanisms in plants against various bacterial and fungal diseases (Mukhtar *et al.*, 2019).

Plants treated with *T. viride* or *B. subtilis* combined with microbial fertilizer NPK develop more rapidly in greenhouse environments. Bacterial isolates of *Micrococcus* species, *Mycobacterium* species, *Escherichia coli*, *S. marcescens*, *B. subtilis*, *P. aeruginosa*, and *Sarcina* species significantly decreased the number of galls, developmental stages, and egg masses in eggplant roots infected with the root-knot nematode (*M. incognita*) in a greenhouse, according to Al-Shalaby and Sedik (2008).

Conversely, Wani *et al.* (2016) showed that the *Azotobacteria* genus synthesizes chemicals similar to GA, cytokinins, and auxins; these growth materials are the main factors controlling the accelerated growth. It promotes nutrient uptake, protects plants from phytopathogens, feeds rhizospheric microorganisms, and ultimately increases biological nitrogen fixation. These hormones, which come from the root surface or rhizosphere, have an impact on the growth of higher plants that are closely related.

Our results showed that the highest percentages in plant growth parameters were recorded by all dual treatments that included NPK microbial fertilizer. These findings concur with those of Estiyar *et al.* (2014), who found that the treatment of *Azotobacter* similarly enhanced the number of branches, pods per plant, and 1000 grain weight. In addition, treatment with fungus and bacteria concurrently had a synergistic impact and significantly increased these parameters which is in consistent with Messele *et al.* (2017) who found that the growth parameters of pepper plants considerably increased following the dual inoculation of *Bacillus* and *Trichoderma* spp. Moreover, the combination of *Trichoderma* and *Bacillus* spp. produced the best growth metrics, fruit yields, and plant nutritional content in pot trials compared to single inoculation (Morsy *et al.*, 2009).

Frequent studies have shown that the *Azotobacter*, *Bacillus*, and *Pseudomonas* species are members of the class of microorganisms known as plant growth-promoting rhizobacteria (Sivasakthi *et al.*, 2014; Jnawali *et al.*, 2015; Romero-Perdomo *et al.*, 2017). Hashem *et al.* (2019) further reported that these species were able to protect plants from pathogens and stressors, as well as lengthen their lifespan and secrete metabolites and a variety of hydrolytic enzymes (cellulases, β-glucanases, and proteases) that were involved in the promotion of plant growth. *Bacillus* is known to synthesize a wide range of secondary metabolites, hormones, enzymes that break down cell walls as well as antioxidants which help the plant to defend itself against pathogen invasions. Different *Bacillus* species that are capable of producing siderophores have been found by Sarwar *et al.* (2020) as having the ability to boost the bioavailability of iron in soil at least by 69%. According to Adam *et al.* (2014), *Bacillus subtilis* Sb4-23 activated generated systemic resistance, which decreased nematode activity in tomatoes.

Photosynthesis is one of the most crucial processes in a plant. The latest study's findings revealed a serious lack of pigments needed for photosynthesis as a result of nematode infection; this is because the plant is unable to absorb light and its chlorophyll pigments were broken down. This implies that photosynthesis had been reduced or prevented because the plant was unable to absorb sunlight (Sharma *et al.* 2012; Gámez-Arcas *et al.* 2021). It's important to note that using *T. viride* in combination with NPK as a typical biofertilizer resulted in a distinct and appreciable improvement in chlorophyll pigments. This supports the concept of employing microbial bioagents in conjunction with plant growth stimulants to cure nematode infection-related damage.

In our work, it was observed total carbohydrates and protein increase to the higher levels upon the application of various microbial bioagents compared to the untreated plants which agree with El-Deriny *et al.* (2022), who discovered that nematode infection caused eggplant plants to have lower overall sugar levels. Conversely, and consistent with our findings, applying the endophytic fungus *Aspergillus ochraceus* to barely plants resulted in notable increases in their protein and sugar content (Badawy *et al.*, 2021; Alhaithloul *et al.*, 2019). According to certain research (Keunen *et al.*, 2013; Abdel Latef *et al.*, 2021), the buildup of carbohydrates in plant tissues may boost the synthesis of antioxidants and provide protection against both biotic and abiotic stressors.

Intracellular oxidative stress brought on by biotic and abiotic conditions resulted in significant disruption inthe plant cell and an increase in MDA level (Dallagnol *et al.* 2011). However, when various microbial bioagents were added to the impacted plants, the level of MDA significantly decreased. This can be explained by the bioagents' capacity to increase antioxidants that are involved in defense and induce systemic resistance, which lowers oxidative stress in cells (Badawy *et al.*, 2021).

Infection has been linked to a number of antioxidant defense enzymes including catalase, peroxidase, polyphenol oxidase and superoxide dismutase (Sofy et al. 2020). In order to defend it, infected plants increased their enzymatic activity when treated with microbial fertilizer and bioagent. The results of this investigation showed that eggplants infected with root knot nematodes have higher proline content.

Plants collect osmolytes to scavenge reactive oxygen species and to cope with various environmental challenges, such as proline, which functions as an osmo-regulator (Li *et al*. 2017).

Conclusions and Recommendation

An efficient strategy that can be used to enhance plant development and guarantee plant defense against various plant diseases is the employment of beneficial microorganisms that promote plant growth. Our findings emphasized that the presence of PGPR and *Trichoderma* spp. promoted plant development and suppressed plant parasitic nematodes that maximize the potential of fungal and bacterial interactions with plants for ecological remediation in the further studies.

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Novelty Statement

Using PGPR and Trichoderma spp. to improve plant growth and control plant parasitic nematodes, thus reducing the use of chemical nematicides.

Author's Contributions

Conceptualization: El-Deriny, M.M. and Ibrahim, D.S.S.; Methodology: El-Deriny, M.M.; Ibrahim, D.S.S., Wahdan, R.H. and Fouad, M.S.**;** Reviewing: El-Deriny, M.M.; Ibrahim, D.S.S. and Fouad, M.S.; Statistical analysis: Fouad, M.S. and El-Deriny, M.M.; Editing and Writing - original draft: El-Deriny, M.M.; Ibrahim, D.S.S., Wahdan, R.H. and Fouad, M.S.

Conflict of interests
There are no conflicts of interest to declare.

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