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# Efficacy of *Paecilomyces, Bacillus* and *Trichoderma* as Biocontrol Agents Against *M. javanica* on Pepper under Geenhouse Conditions

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## Abstract

The aim of this work is to study the effect of *Paecilomyces*, *Bacillus* and *Trichoderma* as a biocontrol against *M. javanica* and treatment times of biological control application on pepper under greenhouse conditions. A total of 75 plastic pots were divided into seven experimental groups. The first group was kept without infection as healthy control. Two weeks later, the pots of the other groups were inoculated with roughly 5000 eggs and j2 / pot. The second group, which acted as an infected control, was kept without treatment The third to sixth groups were treated with bioagents (*Paecilomyces* (P.), *Bacillus* (B.), *Trichoderma* (T.) and *Paecilomyces* + *Bacillus*+ *Trichoderma* (P.B.T.), respectively. The 7$^{th}$ group received Vydate (nematicide). The 3$^{rd}$ to 6$^{th}$ groups were divided into three subgroups which received biological treatments at three phases (Before- With- After) infection. These four biological control treatments induced a significant decrease in disease parameter compared with control. The highest reduction% in galls, egg masses and number of J2/250 cm3 of soil was obtained with Vydate application followed by those obtained with P.B.T when applicated before-, with- and after-infection then *Paecilomyces* when applicated before- and with-infection than the other treatments. All of the tested biological controls caused significant increases in the growth parameter of pepper (GPP). Highest GPP was obtained with biological control application before- and with-infection.

***Keywords:*** *Biocontrol; nematode; pepper.*

## Introduction

Chili (*Capsicum annuum*) is a popular commercial crop farmed all over the world and is high in protein, vitamin C, ascorbic acid, and other nutrients. In chili, plant parasitic nematodes are responsible for several of crop losses (Khan *et al.,* 2012).

Root-knot nematodes (RKN) are hardy pests that damage a wide range of food and industrial crops worldwide. RKN difficulties, susceptibility of current crop varieties (Stirling 2006), and the lack of cost-effective control options make it difficult for growers to prevent crop losses.

Root-knot nematode causes stunted development, decreased crop quality and production, and decreased resilience to a variety of other pressures (Borah *et al.,* 2018; Kepenekci *et al.,* 2018). They cause extensive damage to a variety of crops, including tomato, cucumber, cotton, carrot, pepper, rice, watermelon, eggplant, potato, and others, leading in severe yield losses. Root knot nematode infection causes a variety of biochemical changes in plants, including changes in amino acid and organic acid levels, as well as a reduction in chlorophyll content (Saikia *et al.,* 2013). There are over 80 different infectious species, while three of them (*Meloidogyne arenaria, M. incognita, and M. javanica*) are of essential agronomic importance because they are the only ones that cause at least 90% of crop loss and account for 5% of worldwide crop loss. Furthermore, because of their widespread dispersion, they are highly successful plant parasites (Castagnone-Sereno 2002).

The extensive use of chemically derived inorganic fertilizers and insecticides has caused a significant environmental damage (Hermosa *et al*., 2012; Ansari and Khan, 2012a, b; Ansari and Mahmood, 2017a). As a result of the grave dangers posed by chemical control tactics, researchers are working to develop nonchemical and environmentally friendly management strategies for RKN control (Huang *et al.,* 2016). As a result, scientists have concentrated their efforts on developing various phytonematode management solutions. Furthermore, numerous biological and ecological processes take place in the rhizosphere, which surrounds the plant roots (Bais *et al.,* 2006).

Plant nematode biological control is particularly important because it is thought to be inexpensive, accessible, and ecologically friendly (Ansari and Khan 2012a, b; Ansari and Mahmood 2017b, 2019 a, b). Not only do different *Trichoderma* species have different nematode antagonism strategies, but they can also permeate the rhizosphere and plant roots, allowing them to enhance plant growth.

In one study, the efficacy of *Bacillus subtilis* and *Paecilomyces lilacinus* to diminished *Meloidogyne incognita* on tomato in pots with sterilized soil was tested (Gautam *et al.,* 1995). The microorganisms increased plant height and weight while lowering the gall numbers, females, eggs, and J2, either alone or in combination. The combination of these two biocontrol agents, on the other hand, lowered nematode populations more effectively than each agent alone. Individual administrations resulted in some increases in plant height and weight, but the combination had no overall effect on plant vigor when compared to *P. lilacinus* alone.

These studies frequently reveal increased activity when mixed microorganisms are used. On potted papaya in steamed soil, the beneficial fungus *P. lilacinus* and *Trichoderma harzianum* were used to control *M. incognita* and *Fusarium solani* (Khan *et al.,* 1997). Each biocontrol product, when used alone, improved plant vigor, reduced nematode levels and root rot occurrence. The combination application, on the other hand, was significantly more effective. The objectives of this study were to determine the effectiveness of *Paecilomyces, Bacillus*, and *Trichoderma* as biocontrol agents against *M. javanica*, as well as plant growth promoting when to treat pepper with biological control in greenhouse conditions.

**Materials and Methods**

The experiment was conducted in the Department of Plant Pathology Faculty of Agriculture, Damanhour University from April to August 2018, and it was performed twice. The objectives of this paper were just how *Paecilomyces, Bacillus, and Trichoderma* working as biocontrol agents against *M. javanica* and times of biological control application on pepper in a greenhouse condition.

**- Root-knot nematode culture and inoculum preparation***:*

*Meloidogyne javanica* Treub (Chitwood), was obtained from the Plant Pathology Department, Faculty of Agriculture, Damanhour University. The RKN species was grown as a defined population on tomato. To establish a culture of this worm species, single egg-masses of adult females were identified by morphological traits of the female perineal patterns (Taylor and Sasser, 1978) and cultured on tomato in a greenhouse. RKN eggs were collected from contaminated tomato roots using a 0.5 percent sodium hypochlorite (NaOCl) solution, according to Hussey and Barker (1973).

Cultures of *Bacillus, Trichoderma harzianum* and *M. javanica* Treub (Chitwood), was obtained from the Plant Pathology Department, Faculty of Agriculture, Damanhour University and *Paecilomyces* were obtained from Assuit University. The RKN species was grown as a defined population on tomato. To establish a culture of this worm species, single egg-masses of adult females were identified by morphological traits of the female perineal patterns (Taylor and Sasser, 1978) and cultured on tomato in a greenhouse. RKN eggs were collected from contaminated tomato roots using a 0.5 percent sodium hypochlorite (NaOCl) solution, according to Hussey and Barker (1973).

Green bell pepper / chile campana plants (*Capsicum annuum* L.) were obtained from the Horticulture Research Division, Ministry of Agriculture, Egypt.

**Greenhouse experiment**

A total of 75 plastic pots, a diameter of 20 cm and a depth of 15 cm, were divided into seven experimental groups including 15 single treatments with five pots for each. Pots were supplied with 2.5 kilograms soil mix of sterilized sand: clay soil (3:1, v:v), and were transplanted with 4-weeks old pepper seedlings. The first group was kept without infection with *M. javanica* and served as a non-inoculated untreated control (healthy control), whereas pots in the other groups (groups 2–7) were inoculated by *M. javanica* at two weeks after transplantation with roughly 5000 eggs and J2 / pot. The second group was maintained untreated with biological and chemical nematicides and acted as an infected untreated control group. The biocontrol agents, *Paecilomyces (P.), Bacillus (B.), Trichoderma (T.),* and *Paecilomyces + Bacillus+ Trichoderma* (P.B.T.) were given to the third to sixth groups at a rate of 1000 µg/ml/pot. Also, the 3$^{rd}$ to 6$^{th}$ groups were divided into three subgroups (each with 5 pots) which received the biocontrol agents at three phases, i.e. treatment was conducted before infection by 2 days, the same time with infection, after infection by 2 days. The 7$^{th}$ group, given the nematicide, Vydate G10 (oxamyl) which was applied at 2 g / pot at the same time of nematode inoculation and served as positive control. Each treatment was repeated five times and a completely randomized design has been used.

**Assessment of Root-knot disease parameter:**

Pepper plants from both the non-inoculated control and infected were harvested 60 days following nematode inoculation. Roots and shoots were harvested and rinsed under running tap water. Galled roots were placed in an aqueous solution of phloxin B stain (0.15 gm/ $cm^3$ tap water) for 15-20 minutes the remaining discoloration was then rinsed away using running tap water. Galls numbers, egg-masses, eggs / plant and number of $J_2$ / 250 $cm^3$ soil were determined. The following formulas were used to calculate the increase or reduction (percentage) of nematode parameters and the nematode reproduction factor (RF):

Reproduction factor (RF) = Pf/ Pi, where,

Pf= Final nematode population = number of eggs /plant + number of $J_2$/pot at the harvest time

Pi= initial nematode population = 5000 eggs and $J_2$.

**Assessment of pepper growth parameters:**

The upper mentioned pepper plants were also subjected to GPP determinations, i.e. root fresh weight (RFW), root dry weight (RDW), root length, shoot fresh weight (SFW), shoot dry weight (SDW) and shoot length.

**Statistical analysis**

Using the SPSS system (2006), data were statistically analyzed using analysis of variance (ANOVA) according to Snedecor and Chochran (1982). Duncan's New Multiple Range Test was used to assess the variations in means (Duncan, 1955).

**Experimental Results**

**1. Nematicidal activity of biocontrol agents under greenhouse conditions:**

Table 1 summarizes the impact of BC; *Paecilomyces, Bacillus, Trichoderma, and Paecilomyces + Bacillus+ Trichoderma* on disease parameters of *M. javanica* on pepper roots, 60 days after transplanting. In comparison to the control, these four biological control treatments reduced the gall numbers, egg masses, and eggs per plant, as well as the J2 number. Vydate treatment resulted in the largest percent reduction in all nematode parameters with (100.0%) in galls, (100.0%) in egg masses, (100.0%) in number of J2/250 $cm^3$ soil, (100.0%) in eggs and (100.0%) in RF followed by those obtained with P.B.T applications with (72.07%) in galls, (75.36%) in egg masses, (79.96%) in number of J2/250 $cm^3$ soil, (80.46%) in eggs and (80.45%) in RF then *Paecilomyces* application treatment with (58.29%) in galls, (68.13%) in egg masses, (72.33%) in number of J2/250 $cm^3$ soil, (73.41%) in eggs and (73.39%) in RF than *Bacillus* and *Trichoderma* application treatments with (39.75; 35.38%) in galls, (54.76; 53.51%) in egg masses, (61.37; 53.18%) in number of J2/250 $cm^3$ soil, (60.45; 59.72%) in eggs and (60.47; 59.58%) in RF, respectively.

The effect of application time of biological control application (Before- With- After) infection, on the number of galls, egg masses and eggs of *M. javanica* on pepper roots, and number of J2 in 250 g soil after 60 days of transplanting is summarized in Table 2. The highest percentage reduction in the all nematode parameters was obtained with biological control application before infection with (63.46%) in galls, (71.39%) in egg masses, (78.88%) in number of J2/250 $cm^3$ soil, (76.48%) in eggs and (76.53%) in RF followed by those obtained with biological control application with or after infection with (46.66; 44.00%) in galls, (60.02; 57.42%) in egg masses, (61.41; 59.84%) in number of J2/250 $cm^3$ soil, (64.18; 64.87%) in eggs and (64.12; 64.77%) in RF, respectively.

**Table 1. Effect of biocontrol agents on disease parameters of pepper plants infected with *M. javanica* and reduction %, under greenhouse conditions.**

| Treatment | G | *R* | EM | *R* | $J_2$ | R | Eggs | *R* | RF | *R* |
|---|---|---|---|---|---|---|---|---|---|---|
| **Untreated healthy control** | 0.00f | - | 0.00e | - | 0.00f | - | 0.00e | - | 0.00e | - |
| **Untreated inoculated control** | 362a | - | 342a | - | 516a | - | 143806a | - | 29.79a | - |
| *Pacylomyces***(P)** | 151d | 58.29 | 109c | 68.13 | 143d | 72.33 | 38239c | 73.41 | 7.93c | 73.39 |
| *Bacillus* **(B)** | 218c | 39.75 | 155b | 54.76 | 199c | 61.37 | 56875b | 60.45 | 11.77b | 60.47 |
| *Trichoderma* **(T)** | 234b | 35.38 | 159b | 53.51 | 241b | 53.18 | 57923b | 59.72 | 12.07b | 59.58 |
| **P+B+T.** | 101e | 72.07 | 84.2d | 75.36 | 103e | 79.96 | 28103d | 80.46 | 5.82d | 80.45 |
| **Vydate (nematicide)** | 0.00f | 100 | 0.00e | 100 | 0.00f | 100 | 0.00e | 100 | 0.00e | 100 |
| **SEM** | - | - | - | - | - | - | - | - | - | - |
| **Sig.** | 0.004 | - | 0.001 | - | 0.005 | - | 0.009 | - | 0.005 | - |

G= Number of galls; EM= Egg masses; $J_2$= number of J2/250 $cm^3$ soil; RF= Reproduction factor; R% = Reduction (%); *P.B.T.=Paecilomyces +Bacillus+ Trichoderma;* SEM=Stander error of means; Sig= Significant; Means with the same letter (s) in each column are not significantly different at P=0.05.

**Table 2. Effect of certain biocontrol agents, and their time of application, before, with, or after inoculation with M. javanica, on disease parameters of infected pepper and reduction% (R) under greenhouse conditions.**

| Time | G | *R* | EM | *R* | $J_2$ | R | Eggs | *R* | RF | *R* |
|---|---|---|---|---|---|---|---|---|---|---|
| **Untreated healthy control** | 0.00d | - | 0.00d | - | 0.00d | - | 0.00d | - | 0.00d | - |
| **Untreated inoculated control** | 362a | - | 342a | - | 516a | - | 143806a | - | 29.79a | - |
| **Before** | 132c | 63.46 | 97.7c | 71.39 | 109c | 78.88 | 33827c | 76.48 | 6.98c | 76.53 |
| **With** | 193b | 46.66 | 137b | 60.02 | 199b | 61.41 | 51510b | 64.18 | 10.70b | 64.12 |
| **After** | 203b | 44.00 | 145b | 57.42 | 207b | 59.84 | 50517b | 64.87 | 10.51b | 64.77 |
| **Vydate** | 0.00d | 100 | 0.00d | 100 | 0.00d | 100 | 0.00d | 100 | 0.00d | 100 |
| **Sig.** | 0.001 | - | 0.001 | - | 0.001 | - | 0.001 | - | 0.001 | - |

G= Number of galls; EM= Egg masses; $J_2$= number of J2/250 $cm^3$ soil; RF= Reproduction factor; R% = Reduction (%); *P.=Paecilomyces ; B.=Bacillus*; T.=*Trichoderma*; *P.B.T.=Paecilomyces +Bacillus+ Trichoderma;* SEM=Stander error of means; Sig= Significant; Means with the same letter (s) in each column are not significantly different at P=0.05.

Effect of biocontrol agent applications and their interaction with time of application on root-knot disease parameters of pepper plants inoculated with *M. javanica* is shown in Table 3. Interaction between biological controls and times of biological control application induced significant decrease in galls and J2 number and egg masses compared with infected control. The highest percentage reduction in galls, egg masses and number of J2/250 $cm^3$ soil was obtained with Vydate applications followed by those obtained with P.B.T when applicated before-, with- and after-infection then *Paecilomyces* when applicated before- and with-infection than other treatments. On the other hand, the interaction between biological controls and times of biological control application and Vydate caused non-significant decreases in eggs and RF of pepper.

**Table 3. Effect of certain biocontrol agents, and their time of application, before, with, or after inoculation with *M. javanica,* on root-knot disease parameters of pepper, 60 days after inoculation under greenhouse conditions.**

| Treatments | | Number of galls | Reduction (%) | Eggmasses | Reduction (%) | Number of J2* | Reduction (%) | Eggs | Reduction (%) | RF** | Reduction (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Untreated healthy control** | | 0.00j | - | 0.00h | - | 0.00k | - | 0.00 | - | 0.00 | - |
| **Untreated inoculated control** | | 361.9a | - | 341.5a | - | 515.7a | - | 143806 | - | 29.79 | - |
| ***P.*** | **Before** | 100.1h | 72.34 | 72.75f | 78.70 | 88.01i | 82.93 | 26544 | 81.54 | 5.49 | 81.57 |
| | **With** | 158.8f | 56.13 | 114.7d | 66.43 | 118.3h | 77.06 | 43626 | 69.66 | 8.90 | 69.82 |
| | **After** | 194.0d | 46.39 | 139.2c | 59.26 | 221.8d | 56.98 | 44547 | 69.02 | 9.35 | 68.77 |
| ***B.*** | **Before** | 172.2e | 52.42 | 131.1cd | 61.60 | 154.2f | 70.10 | 42949 | 70.13 | 8.96 | 70.13 |
| | **With** | 232.6c | 35.74 | 174.2b | 48.99 | 185.6e | 64.02 | 71987 | 49.94 | 14.77 | 50.24 |
| | **After** | 249.4b | 31.08 | 158.2b | 53.69 | 257.9c | 50.00 | 55689 | 61.27 | 11.65 | 61.04 |
| ***T.*** | **Before** | 193.1d | 46.65 | 140.7c | 58.81 | 153.7f | 70.21 | 50306 | 65.02 | 10.37 | 65.13 |
| | **With** | 258.5b | 28.58 | 167.5b | 50.96 | 360.3b | 30.14 | 60167 | 58.16 | 12.75 | 57.57 |
| | **After** | 250.1b | 30.91 | 168.2b | 50.77 | 210.5d | 59.18 | 63295 | 55.99 | 13.08 | 56.05 |
| ***P.B.T.*** | **Before** | 63.63i | 82.42 | 46.32g | 86.44 | 39.87j | 92.27 | 15510 | 89.21 | 3.18 | 89.28 |
| | **With** | 122.4g | 66.19 | 89.89e | 73.68 | 132.0gh | 74.41 | 30261 | 78.96 | 6.32 | 78.86 |
| | **After** | 117.3g | 67.61 | 116.3d | 65.96 | 138.1fg | 73.22 | 38538 | 73.20 | 7.98 | 73.20 |
| **Vydate** | | 0.00j | 100 | 0.00h | 100 | 0.00k | 100 | 0.00 | 100 | 0.00 | 100 |
| **SEM** | | 3.65 | - | 4.43 | - | 4.74 | - | 3155 | - | 0.633 | - |
| **Sig** | | 0.001 | - | 0.002 | - | 0.001 | - | 0.068 | - | 0.084 | - |

*Number of J2/250 $cm^3$ soil; **RF= Reproduction factor; *P.=Paecilomyces; B.=Bacillus*; T.=*Trichoderma*; *P.B.T.=Paecilomyces +Bacillus+ Trichoderma;* SEM=Stander error of means; Sig= Significant. Means with the different letter (s) in each column are significantly different at P=0.05.

## 2- Effect of biocontrol agents on growth characteristic of pepper:

All the BCs tested resulted in significant increase in pepper SFW and SDW (Table 4). This biological controls also showed plant growth increase activity similar to Vydate, with the exception of *Trichoderma*, which was lower than Vydate. Furthermore, the tested biological controls and Vydate caused significant increase in the RFW and RDW, as well as the root length of pepper, as compared to the infected control group, with the exception of *Trichoderma*, which had the same root length as the infected control group.

Table 5 summarize the influence of biological control application (Before- With- After) infection on Pepper transplanting growth characteristics. All times of biological control application (Before- With- After) infection caused significant increase in GPP. The highest in the SFW and SDW, the RFW and the root length of pepper was obtained with biological control application before- and with- infection followed by those obtained with biological controls application after infection compared with infected control.

The effect of interaction between biological control and the application times of biological control application on GPP plants infected with *M. javanica* is summarized in Table 6. The interaction between biological controls and the application times of biological control application and Vydate caused non-significant increase in the GPP plants compared with infected control.

**Table 4. Effect of biological control on GPP plants infected with *M. javanica* and increasing % (I), under greenhouse conditions.**

| Treatment | Shoot weight (g) | | | | Shoot Length | | Root weight (g) | | | | Shoot Length | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fresh | *I* | Dry | *I* | cm | *I* | Fresh | *I* | Dry | *I* | cm | *I* |
| **Untreated healthy control** | 50.68a | - | 11.51a | - | 51.68 | - | 15.32a | - | 5.20a | - | 22.60a | - |
| **Untreated inoculated control** | 29.78c | - | 5.83c | - | 31.71 | - | 10.04c | - | 3.31c | - | 16.15b | - |
| **P.** | 48.90a | 64.16 | 11.42a | 95.76 | 44.26 | 39.57 | 14.75a | 46.90 | 4.82ab | 45.66 | 21.30a | 31.94 |
| **B.** | 48.56a | 63.03 | 11.43a | 96.03 | 43.91 | 38.48 | 13.90ab | 38.44 | 4.34b | 31.13 | 20.65a | 27.91 |
| **T.** | 43.95b | 47.55 | 8.31b | 42.54 | 44.20 | 39.39 | 12.66b | 26.09 | 4.19b | 26.60 | 19.17b | 18.69 |
| **P.B.T.** | 50.91a | 70.91 | 11.19a | 91.94 | 46.18 | 45.63 | 14.59a | 45.23 | 5.18a | 56.54 | 22.72a | 40.74 |
| **Vydate** | 50.70a | 70.21 | 11.24a | 92.81 | 46.98 | 48.15 | 14.75a | 46.88 | 5.08a | 53.62 | 22.09a | 36.82 |
| **SEM** | | - | | | - | | | - | | - | | - |
| **Sig.** | 0.001 | - | 0.005 | | 0.086 | | 0.001 | - | 0.002 | - | 0.001 | - |

*P.=Paecilomyces; B.=Bacillus*; T.=*Trichoderma*; *P.B.T.=Paecilomyces +Bacillus+ Trichoderma;* SEM=Stander error of means; Sig= Significant; *I* = increase (%); Means with the same letter (s) in each column are not significantly different at P=0.05.

**Table 5. Effect of the application times of biological control application (before-with- after) infection on growth parameters of pepper infected with *M. javanica* and increasing % (I), under greenhouse conditions.**

| Time | Shoot weight (g) | | | | Shoot Length | | Root weight (g) | | | | Shoot Length | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fresh | *I* | Dry | *I* | cm | *I* | Fresh | *I* | Dry | *I* | cm | *I* |
| **Untreated healthy control** | 50.68a | - | 11.51a | - | 51.68a | - | 15.32a | - | 5.20a | - | 22.60a | - |
| **Untreated inoculated control** | 29.79c | - | 5.83c | - | 31.71d | - | 10.04c | - | 3.31c | - | 16.15c | - |
| **Before** | 51.53a | 72.99 | 11.48a | 96.94 | 45.30bc | 42.86 | 14.61a | 45.53 | 4.83ab | 45.96 | 21.81a | 35.09 |
| **With** | 49.85a | 67.35 | 10.85a | 86.11 | 45.25bc | 42.68 | 14.61a | 45.48 | 4.79ab | 44.87 | 21.91a | 35.72 |
| **After** | 42.86b | 43.89 | 9.43b | 61.65 | 43.37c | 36.76 | 12.70b | 26.49 | 4.27b | 29.12 | 19.16b | 18.63 |
| **Vydate** | 50.70a | 70.21 | 11.24a | 92.81 | 46.98b | 48.15 | 14.75a | 46.88 | 5.08a | 53.62 | 22.09a | 36.82 |
| **SEM** | | - | | | - | | | - | | - | | - |
| **Sig.** | 0.001 | - | 0.003 | | 0.038 | | 0.002 | - | 0.01 | - | 0.004 | - |

*P.=Paecilomyces; B.=Bacillus*; T.=*Trichoderma*; *P.B.T.=Paecilomyces +Bacillus+ Trichoderma;* SEM=Stander error of means; Sig= Significant; *I* = increase (%); Means with the same letter (s) in each column are not significantly different at P=0.05.

**Table 6. Effect of certain biocontrol agents, and their time of application, before, with, or after inoculation with *M. javanica*, on root-knot GPP, 60 days after inoculation under greenhouse conditions.**

| Treatment | | Shoot weight (g) | | | | Shoot Length | | Root weight (g) | | Shoot Length | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fresh | *I* | Dry | *I* | cm | *I* | Fresh | *I* | Cm | *I* |
| UIC | | 50.68 | - | 11.51 | - | 51.68 | - | 15.32 | - | 22.60 | - |
| IC | | 29.79 | - | 5.83 | - | 31.71 | - | 10.04 | - | 16.15 | - |
| P. | Before | 50.33 | 68.95 | 12.52 | 114.70 | 43.03 | 35.70 | 15.18 | 51.15 | 21.88 | 35.51 |
| | With | 52.81 | 77.30 | 11.29 | 93.60 | 46.84 | 47.72 | 15.91 | 58.42 | 22.77 | 41.05 |
| | After | 43.56 | 46.24 | 10.44 | 78.98 | 42.90 | 35.28 | 13.17 | 31.12 | 19.26 | 19.27 |
| B. | Before | 52.49 | 76.21 | 11.99 | 105.70 | 44.03 | 38.84 | 14.40 | 43.35 | 21.53 | 33.33 |
| | With | 49.63 | 66.60 | 12.02 | 106.13 | 44.71 | 40.98 | 13.88 | 38.20 | 21.05 | 30.39 |
| | After | 43.58 | 46.29 | 10.28 | 76.26 | 43.01 | 35.62 | 13.43 | 33.76 | 19.37 | 20.00 |
| T. | Before | 48.00 | 61.14 | 9.55 | 63.79 | 45.90 | 44.74 | 13.17 | 31.11 | 19.35 | 19.83 |
| | With | 45.40 | 52.41 | 8.70 | 49.22 | 43.83 | 38.23 | 13.11 | 30.55 | 19.87 | 23.05 |
| | After | 38.45 | 29.08 | 6.68 | 14.60 | 42.88 | 35.22 | 11.70 | 16.53 | 18.30 | 13.31 |
| P.B.T. | Before | 55.30 | 85.65 | 11.87 | 103.57 | 48.26 | 52.17 | 15.72 | 56.52 | 24.49 | 51.67 |
| | With | 51.56 | 73.10 | 11.40 | 95.50 | 45.61 | 43.82 | 15.54 | 54.75 | 23.96 | 48.38 |
| | After | 45.86 | 53.97 | 10.31 | 76.75 | 44.68 | 40.90 | 12.50 | 24.43 | 19.72 | 22.16 |
| Vydate | | 50.70 | 70.21 | 11.24 | 92.81 | 46.98 | 48.15 | 14.75 | 46.88 | 22.09 | 36.82 |
| SEM | | 1.061 | - | 0.403 | - | 0.930 | - | 0.501 | - | 0.756 | - |
| Sig | | 0.361 | - | 0.735 | - | 0.165 | - | 0.285 | - | 0.678 | - |

UIC =uninfected control; IC =infected control; *P.=Paecilomyces; B.=Bacillus*; T.=*Trichoderma*; *P.B.T.=Paecilomyces+Bacillus+ Trichoderma;* SEM=Stander error of means; Sig= Significant; *I* = increase (%) Means with the same letter (s) in each column are not significantly different at P=0.05.

## Discussion

Our results agree with Hermosa *et al*. (2012) and Mukherjee *et al.* (2012) who found that Trichoderma species have good impacts on secondary root proliferation, leaf area, shoot length and SDW. Also, Naserinasab *et al.* (2011) who reported that *Trichoderma harzianum* BI was used as a biocontrol for *M. javanica* by direct parasitism, inhibiting egg hatching, and the formation of bioactive compounds that are fatal to *M. javanica* in tomato fields. Moreover, Khalil *et al.* (2012) who found that the *Paecilomyces lilacinus* product was the best treatment in suppressing the root-knot populations in the soil with (85.2%), followed by those with *B. subtilis and B. thuringiensis* with 82.6 and 80.5% reduction, respectively. Also, *P. lilacinus* increased the shoot length and fresh weight of the root system by 229.0% and 476.46%, respectively. *Bacillus thuringiensis* increased shoot weight and root length of tomato.

Biological control of plant nematodes is particularly significant since it is believed to be cheap, affordable, and environmentally benign (Ansari and Mahmood 2017b, 2019a, b).

The nematicidal activity of *P. lilacinus, B. subtilis, Penicillium spp., Trichoderma viride,* and *Glomus fasciculatum* was discovered to be efficient against *M. incognita* (Esnard *et al.,* 1998). Although the leaves number, root and shoot length, and plant height rose in *P. lilacinus*-treated plants, the root galls index and eggs per egg mass in root were dramatically reduced.

*Piriformospora indica*, an endophytic fungus, was found to effectively control RKN infection when paired with two plant growth-promoting rhizobacteria (*Bacillus pumilus* and *Pseudomonas fluorescens*) (Varkey *et al.,* 2018).

The activation of systemic resistance and defense mechanisms in plants infection with nematodes could account for the considerable reduction in root-knot disease (Ma *et al.,* 2017). It also helps with nutrient availability and may act as a biocontrol agent, boosting plant growth and compensating for RKN damage (Sharma and Sharma 2017).

Antimicrobial substances produced by *B. subtilis* include subtillin, bacitracin, bacillomycin, bacillin, and subtenolin (Killani *et al.,* 2011). It has also been proven to produce nematicidal compounds such as, 2-undecanone, 2-nonanone, benzene acetaldehyde, dimethyl disulfide, and decanal, which are antagonistic to RKN egg hatching and J2 (Huang *et al.,* 2010). The carrot output increased by 28.8%, reduced population of nematode by 69.3%, and reduced disease occurrence by 70.2 percent when treated seed by *B. subtilis*-enriched and composts to the soil combined (Rao *et al*., 2017).

*Trichoderma* species have good impacts on secondary root proliferation, leaf area, shoot length, SDW, and crop output (Hermosa *et al*., 2012; Mukherjee *et al.,* 2012). *Trichoderma* spp. are plant growth-promoting fungi (PGPF) that secrete a wide range of stimulating plant growth chemical compounds, such as phytohormones (Doni *et al.,* 2013; Ansari and Mahmood, 2019b).

In addition, *Trichoderma harzianum* BI was used as a biocontrol for *M. javanica* by direct parasitism, inhibiting egg hatching, and the formation of bioactive compounds that are fatal to *M. javanica* in tomato fields (Naserinasab *et al.,* 2011). In a greenhouse test, cucumber plants were also given conidial solutions of *Trichoderma sp*. before and after infection by *M. incognita*. Surprisingly, nematode reproduction was reduced by 50 percent (Mascarin *et al*., 2012). More over half of the juveniles of *M. javanica* were killed by *Trichoderma spp*. culture filtrates, where, *T. viride* S-3-treated plants showed the highest mortality rate (90 %), followed by T. *harzianum*-treated plants (88 %) (Qureshi *et al*., 2012). In addition, in Kenya, *T. asperellum* M2RT4 decreased galls, eggs, and egg-masses in roots of pineapple (Kirigaa *et al*., 2018). Numerous *Trichoderma* species demonstrated greater chitinase activity in terms of both quality and quantity, as well as significant activity against *M. incognita* infested tomato (Sayed *et al*., 2019).

*Trichoderma* species efficiently release various hydrolytic enzymes, such as chitinase (Anand and Reddy 2009), protease, and 1,3-glucanase (Gajera *et al.,* 2012), which cause nematode cell wall breakdown (Cheng *et al*., 2017). *T. viride* also produces several of potent antibiotics, including sesquiterpene heptalic acid, dermadin, trichodermin, and trichoviridin that help reduce plant-parasitic nematodes (Abd-Elgawad and Askary 2020). *Trichoderma* species can attack plant diseases through a variety of ways, including direct parasitism, food competition, antibiosis, enzymatic hydrolysis, and disease resistance (Harman *et al*., 2004; Howell 2003). In the absence of pests and pathogens, plant yield can be increasing by used *Trichoderma spp*. (Sharon *et al.,* 2001; Yedidia *et al.,* 1999).

*Bacillus subtilis and Paecilomyces lilacinus* were investigated for their ability to reduce *M. incognita* on tomato in pots with sterilized soil in one study (Gautam *et al*., 1995). The microorganisms, alone or in combination, enhanced plant height and weight while reducing the gall numbers, eggs, females, and J2. The combination of these two biocontrol agents, on the other hand, lowered nematode populations more effectively than each agent alone. Individual administrations resulted in some increases in plant height and weight, but the combination had no overall effect on plant vigor when compared to *P. lilacinus* alone.

While the biocontrol agents *P. penetrans, P. lilacinus, Talaromyces flavus*, and *B. subtilis* lowered RKN indices in general, Zaki and Maqbool (1991) found that combinations were not more successful than individual application of these biocontrol agents.

These studies frequently reveal increased activity when mixed microorganisms are used. On potted papaya in steamed soil, the beneficial fungus *P. lilacinus* and *Trichoderma harzianum* were used to control *M. incognita* and *Fusarium solani* (Khan *et al*., 1997). Each biocontrol product, when used alone, improved plant vigor, reduced nematode levels, and reduced root rot occurrence. The combination application, on the other hand, was significantly more effective.

Strains combinations have been found to be capable of delivering effective control of a number of diseases on different crop species in studies using treatments including combinations of two or more antagonists. Because strains are frequently paired without consideration of interactions among biocontrol agents, the success of strain combinations cannot always be predicted from individual microbe performance as biocontrol agents. More effort is needed to avoid negative interactions while retaining beneficial interactions arising from biocontrol agent co-application. With a greater understanding of the ecological foundation of the interactions among bacteria used for biocontrol, it is believed that levels of disease-suppressive soil performance can be approximated. When two or more microorganisms are involved, manufacturing and quality control issues are amplified from a commercial standpoint. Specific formulations for numerous microorganisms will be required.

## Conclusion

The present study implies that treatment combination of P.B.T when applicated before-infection was found to be the best treatment as it recorded higher growth parameters with least disease parameter.

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**فعالية استخدام *Paecilomyces* و *Bacillus* و *Trichoderma* كعوامل للمكافحة الحيوية ضد *M. javanica* على الفلفل تحت ظروف الصوبة**

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**الملخص**

تهدف هذه الدراسة إلى دراسة تأثير *Paecilomyces* و *Bacillus* و *Trichoderma* كعوامل مكافحة بيولوجية ضد النيماتودا *M.javanica* وأوقات تطبيق المكافحة البيولوجية على الفلفل تحت ظروف الصوبة. تم تقسيم إجمالي ٧٥ أصيص إلى سبع مجموعات تجريبية. وتركت المجموعة الأولى بدون عدوى *M. javanica* كمجموعة كنترول غير مصاب. تم عدوى أصص المجموعات الأخرى بحوالي ٥٠٠٠ بيضة ويرقه لـ *M . javanica* / أصيص، بعد أسبوعين من الزراعة. المجموعة الثانية بقيت بدون معاملات بيولوجية وكيميائية بمبيدات النيماتودا كمجموعة كنترول مصاب. حصلت المجموعات من الثالثة إلى السادسة المعاملات البيولوجية *Paecilomyces*، *Bacillus*، *Trichoderma* و *Paecilomyces + Bacillus + Trichoderma* على التوالي، المجموعة السابعة تلقت المبيد النيماتودى Vydate. تم تقسيم المجموعات من ٣ إلى ٦ إلى ثلاث مجموعات فرعية تلقت المعاملات البيولوجية على ثلاث مراحل (قبل - مع - بعد) العدوي. أدت هذه المعاملات الأربعة للمكافحة البيولوجية إلى انخفاض معنوي في الصفات المرضية مقارنة بمجموعة الكنترول. وتم الحصول على أعلى نسبة انخفاض في العقد الجذرية وكتل البيض وعدد اليرقات في ٢٥٠ سم مكعب من التربة باستخدام المعاملة بالمبيد النيماتودي Vydate تليها تلك التي تم الحصول عليها باستخدام خليط من عوامل المكافحة البيولوجية عند تطبيقها قبل ومع الإصابة وبعدها، ثم *Paecilomyces* عند تطبيقها قبل - ومع الإصابة مقارنة مع المعاملات الأخرى. أدت جميع المعاملات البيولوجية المختبرة إلى زيادة كبيرة في صفات النمو في الفلفل. تم الحصول على أعلى صفات نمو للفلفل باستخدام المكافحة البيولوجية قبل ومع العدوى.