Egypt. J. Agronematol., Vol. 23, No.2, PP. 38- 53 (2024)
DOI: 10.21608/EJAJ.2024.376785

Evaluation the Efficacy of some Microbes against Nematode Community (Target and Non-Target Species) Associated with Citrus Trees

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Received: 25 July 2024 *Revised: 28 August 2024* *Accepted: 30 August 2024*

ABSTRACT

A two-season study evaluated five microbes in the nematode community associated with citrus trees under field conditions in Egypt. These microbes were *Trichoderma asperellum*, *T. harzianum*, two strains of the bacteria *Pseudomonas fluorescens*, and the yeast *Rhodosporidium paludigenum*, and the last was not previously studied against plant parasitic nematodes (PPNs). All the tested microbes decreased the number of PPNs under field conditions by the time after application. In addition, *R. paludigenum* was the most effective against the citrus nematode *Tylenchulus semipenetrans*. The reduction percentage in J2s numbers was 59.5 and 60.7% after four weeks of application in the first and second seasons, respectively, while *P. fluorescens* race1 was the most effective against *Xiphinema* spp., with a recorded reduction 75.7 and 77.7% after the same application period in the first and second seasons, successively. On the contrary, no suppressive effect was recorded for the tested microbes on non-target nematodes (NTNs), and their numbers were increased. For example, the *Tylenchus* number increased in *R. paludigenum* application by 52.7% after four weeks of application in the second season. In contrast, *Dorylaimus*, *Mononchus*, and free-living nematodes increased by 69.9, 86.3 and 71.8% in *T. asperellum* application, sequentially, after four weeks of application. On the other hand, the effect of these tested microbes was investigated on *T.semipenetrans* under lab conditions to confirm the effect on J2s activity and eggs hatching. The obtained results were in harmony with the field study. This study aims to investigate the effect of previously tested microbes on beneficial nematodes associated with citrus trees and confirm their biological control role against PPNs with a focus on the promising biocontrol agent (*R. paludigenum*), which was not tested before comprehensively on the nematode community.

***Keywords*:** Biocontrol agents, Citrus, Nematode community, Non-target nematodes, *Mononchus*, PPNs, *Rhodosporidium paludigenum*, *Tylenchulus semipenetrans*

INTRODUCTION

Citrus has economic importance in northern African and southern European countries. This importance has doubled in Egypt, as the citrus crops have priority in export and represent a primary part of the national income from other crops. As a result, Egypt gained the 7th position of the top 10 producing and exporting countries of those crops; about 1.7 million tons of oranges were exported in 2019, equal to 38 % of the world's exports of oranges in 2019 (Anonymous, 2020). In the marketing year 2023/2024 the fresh orange exports reached 2.0 million metric tons (MMT) up from 1.6 MMT in season 2022/2023 (Anonymous, 2023). Unfortunately, citrus trees in Egypt and globally are infected with a large diversity of pests; the danger of plant parasitic nematodes (PPNs) as a major pest of this crop to that they can't be seen with the naked eye and cause significant losses in the crop productivity (Ahuja and

Somvanshi, 2021). The economic losses of crop production due to the infection by (PPNs) were estimated in 2016 at 10-30% (Abd-Elgawad et al., 2016). On the other hand, *Tylenchulus semipenetrans* was the most prominent species infesting citrus groves in Egypt (El-Marzoky et al., 2009, 2018) and can cause the disease, namely slowly decline (citrus dieback), which response to 15-35% of crop yield losses (Afzal et al., 2021).

The use of chemical pesticides was excessed in recent decades for controlling plant parasitic nematodes associated with citrus groves; this excessive use was intense in developing countries, including Egypt (Pretty and Bharucha, 2015), which affected the exports of citrus fruits to the European market. The Egyptian government has passed many laws to rationalize the use of pesticides on local farms, including encouraging farmers to use biopesticides, which raised the export efficiency of its crops, including citrus fruits (Anonymous, 2017).

The bacteria, yeast, and fungi were the safest organisms for controlling soil-borne diseases, including (PPNs). It should be noted that bacteria and fungi were well studied as biocontrol agents for these pests, while yeast was the little (Punja, 1997; Poveda et al., 2020; Lahlali et al., 2022). Beneficial nematodes or not-target nematodes (NTNs) play an essential role in the soil ecosystem. Due to the different natures of these nematode species feeding, their behavior in the soil are varied. Some species decompose the soil organic matter, like free-living nematodes (Yadav et al., 2018; Kekelis et al., 2022). Others feed on bacteria and fungi like *Tylenchus* and *Dorylaimus* (Zheng et al., 2022). Finally, some species play a role as a biocontrol agent against other PPNs, like the predaceous nematodes *Mononchus* (Khan and Kim, 2007; Wang et al., 2015; Kanwar et al., 2021; Ghaderi and Hosseinvand, 2022).

Therefore, this study, focused on the biocontrol role of some microbes on plant parasitic nematodes (PPNs); some of which have been used previously in this mission, and other has not been used yet in controlling programs of PPNs infesting citrus orchards, to reduce the negative impact of the pesticides on the environment with mention to the effect of these biocontrol agents on NTNs associated with the citrus rhizosphere region to obtain any disorder occur in the soil nematode community balance.

MATERIALS AND METHODS

Preparation of the tested microbes

Five microbes were tested in field to study their effects on controlling the nematode associated with the citrus trees. These microbes are marine red yeast *Rhodosporidium paludigenum* (MredY), two species of fungi *Trichoderma asperellum* (Tasp) and *T. harzianum* (Thar), and two strains of the bacteria *Pseudomonas fluorescens* race1 (Pfr1) and race2 (Pfr2); All the tested microbes were attained from the Plant Pathology Department, Faculty of Agriculture, Zagazig University, Egypt. The tested concentrations of these microbes were 2×10^6 CFU/ml in MredY, Tasp, and Thar, while it was 1×10^8 CFU/ml in Pfr1 and Pfr2.

Experimental site

The field experiment was conducted in a citrus orchard cultivated with 15-year-old mandarin trees (*Citrus reticulata*) grafted on sour orange rootstock *C. aurantium*. This site was about six feddans in the Abu-Hammad district, Al-Sharkia Governorate, Egypt. The location coordinates were 30°27'58.6"N 31°40'04.6"E. Seven rows were determined as treatments, and a row separated each. Inside each row, five trees were randomly marked as replicates. The nematicide formulation was granules and applied at the recommended dose around the marked trees. This nematicide was oxamyl (Vydate® 10% G), treated at 150g/ tree (55 kg/ha.). 150 ml of each solution of the tested microbes were applied individually at the abovementioned concentrations

for each tree, while the check treatment was left without any application. All treatments were added around the tree canopy region at the beginning of the day by injection on the upper 25 cm from the surface. The tested materials were added to the marked trees in the first season at the end of February 2020 and repeated in the second season in the same month of 2021. The bio agent's application was replicated weekly for three sequenced weeks from the beginning to ensure their survival and multiplication. The soil samples were collected from the determined trees one, two, and four weeks after the multiplication period. About 300 g of the soil was collected from the four sites around the tree at 20 cm depth, mixed well, then transferred in polyethylene bags to the nematology lab in the Faculty of Agriculture, Zagazig University. The soil samples were transferred in an ice box from the experimental site to the lab and stored in the refrigerator at 10 °C, and the nematode was extracted on the second day by using (Decanting method) a combination of sieves, and the Baermann trays technique (Van Bezooijen, 2006; El-Marzoky, 2019).

The extraction suspension was collected after 24h. of extraction, and the PPNs were morphologically identified under a research microscope using a 1000x magnification power (Mai and Lyon, 1975; Siddiqi, 1986; Van den Berg et al., 2017). Furthermore, NTNs, especially *Tylenchus*, *Dorylaimus*, *Mononchus*, and Free-living nematodes, were recorded to obtain changes in their populations. All species were counted in one ml of the final extraction suspension, and the changes in (PPNs) and (NTNs) were calculated according to equation (1) and equation (2)

(1) The reduction percentage (%) = $\frac{Control-Treatment}{Control} \times 100$

(2) The percentage of increasing (%) = $\frac{Treatment-Control}{Treatment} \times 100$

Citrus nematode juveniles collection and preparation for lab experiment

A lab study was done to obtain the suppressive effect of these microbes on the main nematode species infesting citrus orchards (*T. semipenetrans*). This effect was recorded on juveniles (J2) and eggs. About five kg of the soil sample was collected from heavily infected citrus seedlings. The trees were one-year sour orange seedlings (*C. aurantium*) planted and artificially inoculated with the citrus nematodes J2 six months ago. The J2s were extracted from the soil sample using the same method above.

One ml of nematode suspension was pipetted into a Hawksley counting slide to count the J2s. Each ml of the suspension was estimated to contain about 1500 J2. Next, one ml of the suspension was added to a 15 cm diameter Petri dish and mixed with five ml of the tested microbes at the abovementioned concentrations. Each treatment was replicated five times. The nematicide treatment was prepared using oxamyl 24% SL (soluble liquid) at 1000 ppm concentration (50 ml nematicide + 950 distilled water) and adding five ml to the dishes. The check treatment contained J2 suspension and distilled water, while the other dishes contained the nematodes and the tested materials; all the dishes were put in the incubator at 25 ±2°C and humidity at 75%, and the numbers of inactive J2 (immobile and straight shape) were recorded after 24,48, and 72 hours. The non-active J2 percentage was calculated according to equation (3).

(3) The non-active J2 percentage (%) = $\frac{No.of\ non-active\ J2s}{Initial\ no.\ of\ J2s} \times 100$

Egg collection and preparation

The citrus nematode egg masses were collected from a previously-mentioned heavily-infested citrus root. About ten g of the root was soaked in the tap water for about 5 min. and cut into pieces, each one about 2 cm; these pieces were mixed with 200 ml of the sodium hypochlorite solution (Naocl 0.5%) to dissolve the gelatinous matrix from the egg mass and collect the eggs. The solution was prepared by adding 20 ml of sodium hypochlorite 5% (commercial Clorox®) to 180 ml of distilled water. The mixture was shaken well for three min., and the final suspension was decanted through a 200-mesh sieve nestled upon a 500- mesh sieve. The impurities above the two sieves were immediately washed with light tap water to eliminate the Naocl and maintain egg vitality. The eggs collected on the 500-mesh sieve were transferred with a small quantity of water to a 100 ml beaker; the number of eggs in one ml of the suspension was determined using a research microscope (Hussey and Barker, 1973). About 500 eggs were counted in one ml of the extraction suspension. They added to a 15 cm Petri dishes which contained five ml of the tested materials with the abovementioned concentrations, and each treatment was replicated five times. The dishes were incubated at 25 ±2°C and humidity at 75%. The number of non-hatched eggs was recorded after 24,48,72 h.

The inhibition percentage in egg hatching was calculated according to equation (4):

(4) The egg-hatching inhibition (%) = $\frac{\text{Number of non-hatched eggs}}{\text{Initial no. of eggs}} \times 100$

Statistical analysis

The field experiments were conducted in two successful fruiting seasons and implemented in a randomized complete block design. Data were statistically analyzed using compare means analysis by SPSS (version 16) software and calculating Duncan's multiple range test at probability level ($P \leq 0.05$).

RESULTS AND DISCUSSION

Effect of determined microbes on *T. semipenetrans* under laboratory conditions

In the lab study, the determined microbes were tested on the citrus nematode to confirm their effect on J2 and egg hatching before being applied under field conditions. Furthermore, it was found that all the tested microbes reduced the J2 activity and egg hatching after 24 h. of treatment; these results are shown in Table (1). MredY was the most effective microbe; it reduced J2 activity by 9.0% compared to the nematicide (11.4%). On the other hand, this microbe reduced egg hatching by 44.3% compared to the nematicide (69.0%). The second efficient microbe was Thar which reduced the J2 by 4.0% while the egg hatching reduced by33.7%. Finally, Pfr1 was the least efficient microbe; it decreased the J2 activity by 1.9% and the egg hatching by 17.1%. Thar and Tasp recorded no significant differences in their effect on J2 activity. These percentages increased after 48 h. of treatment; these data were determined in Table (2). The results were in the same trend; MredY had gained the upper hand in the microbial effectivity; it reduced the J2 activity and egg hatching by 21.9 and 24.1% compared with 31.2 and 36.6% in nematicide treatment, sequentially. It could arrange the other microbes descendingly in their effectiveness on J2 activity and egg hatching Thar (14.6 and 18.0%), Tasp (12.7 and 13.9%), Pfr2 (9.0 and 10.7%) and Pfr1 (6.4 and 9.1%), respectively.

Table 1: The suppressive effect of the tested bio-control agents on *Tylenchulus semipenetrans* J2s activity and egg hatching after 24h of the application under laboratory conditions.

Treatments	J2s activity		Egg hatching	
	N. inactive J2s	Immobility percentage (%)	N. non-hatched eggs	Reduction in egg hatching (%)
Check (Untreated)	6.2 f	0.4	35.8 g	7.2
Nematicide	171.2 a	11.4	345.0 a	69.0
Rhodosporidium paludigenum	135.0 b	9.0	221.6 b	44.3
Trichoderma asperellum	51.0 c	3.4	132.8 d	26.5
Trichoderma harzianum	61.4 c	4.0	168.6 c	33.7
Pseudomonas fluorescens race 1	29.4 e	1.9	85.6 f	17.1
Pseudomonas fluorescens race 2	40.4 d	2.6	117.0 e	23.4

*Means in each column followed by the same letter(s) are not significantly different at P ≤0.05 according to Duncan's multiple range test.

Table 2: The suppressive effect of the tested bio-control agents on *Tylenchulus semipenetrans* J2s activity and egg hatching after 48h of the application under laboratory conditions.

Treatments	J2s activity		Egg hatching	
	N. inactive J2s	Immobility percentage (%)	N. non-hatched eggs	Reduction in egg hatching (%)
Check (Untreated)	7.0f	0.4	28.6g	5.7
Nematicide	468.4a	31.2	183.4a	36.6
Rhodosporidium paludigenum	328.6 b	21.9	120.6b	24.1
Trichoderma asperellum	190.6 c	12.7	69.8d	13.9
Trichoderma harzianum	220.0 c	14.6	90.2c	18.0
Pseudomonas fluorescens race 1	97.4 e	6.4	45.6f	9.1
Pseudomonas fluorescens race 2	135.8 d	9.0	53.6e	10.7

*Means in each column followed by the same letter(s) are not significantly different at P ≤0.05 according to Duncan's multiple range test.

Data in Table (3) showed the effectivity of the tested microbes after 72 h, which was the most significant period. The J2 activity was reduced by 62.7, 38.8, 32.0, 28.6, and 7.2% in MredY, Thar, Tasp, Pfr2, and Pfr1, respectively, compared to the nematicide (71.5%). On the other hand, egg hatching was reduced by 17.9, 14.6, 11.1, 9.0, and 17.1% for the abovementioned treatment, sequentially compared to the nematicide 24.4%. All the treatments recorded significant differences in their effect on J2 activity and egg hatching. The obtained results of the tested microbes agreed with many authors e.g., Kumara and Arthurs (2021)

explained that citrus trees are infested with many species of PPNs, including *T. semipenetrans, Radopholus similis, Pratylenchus coffeae,* and *Meloidogyne* spp.

Table 3: The suppressive effect of the tested bio-control agents on *Tylenchulus semipenetrans* J2s activity and egg hatching after 72h of the application under laboratory conditions.

Treatments	J2s activity		Egg hatching	
	N. inactive J2s	Immobility percentage (%)	N.-non-hatched eggs	Reduction in egg hatching (%)
Check (untreated)	21.2g	1.4	21.6g	4.3
Nematicide	1072.6a	71.5	122.0a	24.4
Rhodosporidium paludigenum	940.6b	62.7	89.8b	17.9
Trichoderma asperellum	481.2d	32.0	55.8d	11.1
Trichoderma harzianum	582.2c	38.8	73.2c	14.6
Pseudomonas fluorescens race 1	298.0f	19.8	36.2f	7.2
Pseudomonas fluorescens race 2	429.2de	28.6	45.0de	9.0

*Means in each column followed by the same letter(s) are not significantly different at P ≤0.05 according to Duncan's multiple range test.

They reviewed many microbes on PPNs associated with citrus trees; some of them are fungi like (*Trichoderma* spp., *Purpureocillium lilacinum*, *Pochonia chlamydosporia*, and mycorrhizae, *Glomus* spp.) and bacteria (*Bacillus* spp., *P. fluorescens*, *Streptomyces avermitilis*, and *Pasteuria* spp.). They found all microbes were effective on PPNs and reported they could be used as biocontrol agents. Sahebani and Gholamrezaee (2021) studied the effect of *P. fluorescens* strain CHA0 against root-knot nematode *M. javanica* (endoparasite nematode), which was near *T. semipenetrans* (semi-endoparasite nematode) in it parasitism. They found that this strain of bacteria can reduce nematode disease severity, exemplified by the reduction in the number of galls and egg masses/plant and the number of eggs/individual egg mass.

Effect of tested microbes on nematode associated with citrus trees under field conditions

The effect of the tested microbes on PPNs and NTNs after one, two, and four weeks of treatment of the two tested seasons under field conditions were described in Tables 4 &5 &6 successively. In Table (4) it was found a significant reduction in all treatments in the first season compared to the check treatment after one week of application; the nematicide reduced the nematode numbers by 41.7, 53.8, 15.1, 31.4, 31.8, and 42.8% for species *T. semipenetrans*, *Xiphinema* spp., *Tylenchus* spp., *Dorylaimus* spp., *Mononchus* spp. and free-living nematodes correspondingly. The effect of the tested microbes was arranged descendingly in their effect on *T. semipenetrans* by MredY, Thar, Tasp, Pfr2, and Pfr1.

In contrast, this effect was different on *Xiphinema* spp. as the second major PPNs recorded and arranged by Pfr1, Thar, Tasp, MredY, and Pfr2. On the contrary, the effect of these microbes increased the numbers of NTNs, whereas *Tylenchus* spp. increased by 28.2% in MredY and 18.5 and 2.6% in Tasp and Thar, successively.

Table 4: Effect of biocontrol agents on nematodes associated with mandarin trees after one week of application.

Treatments	Nematode numbers in 250g soil The first season					
	T. semipenetrans	*Xiphinema* spp.	*Tylenchus* spp.	*Dorylaimus* spp.	*Mononchus* spp.	Free-living nematodes
Check (Untreated)	2230.0a (0)	65.0a (0)	74.6c (0)	75.3c (0)	22.0e (0)	93.3d (0)
Nematicide	1300.0e (-41.7)	30.0e (-53.8)	63.3e (-15.1)	51.6f (-31.4)	15.0f (-31.8)	53.3e (-42.8)
Marine red yeast (*Rhodosporidium paludigenum*)	1644.6d (-26.2)	54.3b (-16.4)	104.0a (+28.2)	62.0d (-17.6)	35.3d (+37.7)	106.0c (+11.9)
Trichoderma asperellum	1819.0cb (-18.4)	51.0c (-21.5)	91.6b (+18.5)	162.3a (+53.5)	133.0a (+83.4)	194.3a (+51.9)
Trichoderma harzianum	1692.6d (-24.0)	50.3c (-22.5)	76.6c (+2.6)	93.3b (+19.2)	132.3a (+83.3)	183.6b (+49.1)
Pseudomonas fluorescens race 1	1866.6b (-16.2)	48.3d (-25.6)	74.0c (-0.8)	55.0e (-26.9)	49.6c (+55.6)	183.0b (+49.0)
Pseudomonas fluorescens race 2	1823.3b (-18.2)	56.6b (-12.8)	66.6d (-10.7)	65.3d (-13.2)	63.0b (+65.0)	190.6a (+51.0)
The second season						
Check (Untreated)	2363.0a (0)	76.3a (0)	87.3c (0)	88.3c (0)	25.6d (0)	109.3d (0)
Nematicide	1433.0f (-39.3)	35.3f (-53.7)	74.3d (-14.8)	60.3g (-31.7)	17.6g (-31.1)	62.3e (-42.9)
Marine red yeast (*Rhodosporidium paludigenum*)	1777.6e (-24.7)	63.6c (-16.6)	121.6a (+28.2)	72.6e (-17.7)	41.3f (+37.9)	124.0c (+11.8)
Trichoderma asperellum	1952.0bc (-17.3)	56.6e (-25.7)	107.3b (+18.6)	189.6a (+53.4)	155.6a (+83.5)	227.3a (+51.9)
Trichoderma harzianum	1825.6d (-22.7)	58.6d (-23.1)	89.6c (+2.6)	109.0b (+18.9)	154.6a (+83.4)	214.6b (+49.0)
Pseudomonas fluorescens race 1	1999.6b (-15.3)	56.3e (-26.20)	86.6c (-0.7)	64.3f (-27.1)	58.3c (+56.0)	214.0b (+48.9)
Pseudomonas fluorescens race 2	1956.3bc (-17.2)	66.3b (-13.1)	77.6d (-11.0)	76.3d (-13.5)	73.0b (+64.8)	223.0a (+50.9)

*Means in each separated column followed by the same letter(s) are not significantly different at P ≤0.05 according to Duncan's multiple range test
*values between parentheses refer to percentages of the reduction (-) and the increasing (+) % according to the equations (1 and 2).

In the same trend, the species *Dorylaimus* spp., *Mononchus* spp. and free-living nematodes increased by 53.5, 83.4, and 51.9% in Tasp, while it was recorded in Thar by 19.2, 83.3, and 49.1% for the abovementioned species sequentially. These percentages don't differ significantly in the second season; *T. semipenetrans* reduced by 39.3, 24.7, 22.7, 17.9, 17.2, and 15.3% in nematicide, MredY, Thar, Tasp, Pfr2, and Pfr successively. Moreover, the *Xiphinema* spp. was reduced by 53.7, 26.2, 25.7, 23.1, 16.6, and 13.1% in nematicide, Pfr1, Tasp, Thar, MredY, and Pfr2 sequentially.

On the other hand, the NTNs were significantly increased in the second season; the predacious nematode *Mononchus* spp. They were successively increased by 83.5, 83.4, 64.8, 56.0, and 37.1% in Tasp, Thar, Pfr2, Pfr1, and MredY. Similarly, the free-living nematodes increased by 51.9, 50.9, 49.0, 48.9, and 11.8% in Tasp, Pfr2, Thar, Pfr1, and MredY, sequentially.

After two weeks of application, the reduction effect of the tested microbes on PPNs was increased, and the effect on NTNs was increased, too; this effect was described in Table (5). In the first season, the nematicide decreased *T. semipenetrans* and *Xiphinema* spp. by 54.9 and65.8% sequentially, while the highest reduction percentages in microbes recorded 40.8 in MredY and 54.0% in Pfr1 for the abovementioned species, sequentially. The second effective was Thar which reduced *T. semipenetrans* by 38.8% and *Xiphinema* spp. by 51.4%; finally, Pfr2, the last effective microbe, reduced PPNs by 33.6 and 43.4% for the determined species, respectively.

On the contrary, these microbes increased NTNs associated with the citrus trees. The nematode numbers were increased by 39.5, 7.2, 48.7, and 44.6% in MredY for species *Tylenchus* spp., *Dorylaimus* spp., *Mononchus* spp. and free-living nematodes, successively. Additionally, the most effective treatment-increasing predacious nematode was Tasp, followed by Thar, Pfr2, Pfr1, and MredY with percentages of 83.1, 82.9, 66.6, 62.2, and 48.7%, sequentially. However, the free-living nematodes successively increased by 61.0, 60.5, 59.6, 59.5, and 44.6% in Tasp, Pfr2, Thar, Pfr1, and MredY treatments. The same trend occurred in the second season. MredY was the most effective microbe in reducing citrus nematode by 41.4%, while Pfr1 was superior in reducing dagger nematode by 56.4%. There were significant differences between all treatments in their reduction effect on citrus nematode except between Pfr2 (34.3%) and Tasp (34.1%). At the same time, there are no significant differences between Tasp (53.3%) and Thar (54.0%) in their effect on the dagger nematode. The tested microbes were arranged in descending by their effect on citrus nematode by MredY, Thar, Pfr2, Tasp, and Pfr1. In the same trend, *Tylenchus* spp. increased by 33.7% in MredY. While *Dorylaimus* spp., *Mononchus* spp., and free-living nematodes were increased by 49.7, 80.9, and 58.1% in Tasp. MredY was the least effective microbe on the abovementioned NTN species.

Finally, after four weeks of application, the PPNs were reduced by a percentage exceeding fifty percent in all microbial treatments; this data is explained in Table (6). In the first season, the nematode individuals reduced in oxamyl treatment by 73.0 and 84.7% in *T. semipenetrans* and *Xiphinema* spp., respectively, followed by MredY (59.5%) and Pfr1 (75.7%); in contrast, the lowest effect recorded in Pfr2 were 52.5 and 66.3% for the abovementioned PPNs sequentially. No significant differences were recorded between Thar and Pfr2 on the citrus nematode from one side and between Thar and Pfr1 on the dagger nematode from another side.

Regarding the effect of the tested materials on NTNs, *Tylenchus* spp. was increased by 52.7 and 41.9% in MredY and Pfr2, while, *Dorylaimus* spp. increased between 43.3and 64.2 % in Pfr1and Tasp, successively. The highest increase in *Mononchus* spp. and free-living nematodes

Table 5: Effect of biocontrol agents on nematodes associated with mandarin trees after two weeks of application.

Treatments	Nematode numbers in 250g soil The first season					
	T. semipenetrans	*Xiphinema* spp.	*Tylenchus* spp.	*Dorylaimus* spp.	*Mononchus* spp.	Free-living nematodes
Check (Untreated)	2433.0a (0)	79.0a (0)	90.6e (0)	98.3d (0)	35.0e (0)	116.3e (0)
Nematicide	1095.0f (-54.9)	27.0d (-65.8)	47.3f (-47.7)	38.6e (-60.6)	15.0f (-57.1)	31.3f (-73.0)
Marine red yeast (*Rhodosporidium paludigenum*)	1439.0ed (-40.8)	42.3b (-46.4)	150.0a (+39.5)	106.0c (+7.2)	68.3d (+48.7)	210.3d (+44.6)
Trichoderma asperellum	1618.3c (-33.4)	39.0c (-50.6)	137.6a (+34.1)	206.3a (+52.3)	208.0a (+83.1)	298.9a (+61.0)
Trichoderma harzianum	1487.6d (-38.8)	38.3c (-51.4)	122.6c (+26.0)	137.3b (+28.3)	205.3a (+82.9)	287.9c (+59.6)
Pseudomonas fluorescens race 1	1661.6b (-31.7)	36.3c (-54.0)	120.0c (+24.4)	99.3d (+1.0)	92.6c (+62.2)	287.3c (+59.5)
Pseudomonas fluorescens race 2	1614.0c (-33.6)	44.6b (-43.4)	112.6d (+19.5)	109.3c (+10.0)	105.0b (+66.6)	294.6b (+60.5)
The second season						
Check (Untreated)	2798.6a (0)	95.0a (0)	113.0e (0)	118.0e (0)	45.0e (0)	142.3 (0)
Nematicide	1245.0f (-55.5)	30.6g (-67.7)	53.6f (-52.5)	44.0f (-62.7)	17.3f (-61.4)	35.6d (-74.9)
Marine red yeast (*Rhodosporidium paludigenum*)	1637.6e (-41.4)	48.3bc (-49.1)	170.6a (+33.7)	120.6d (+2.2)	77.6d (-42.0)	239.3a (+40.5)
Trichoderma asperellum	1841.6c (-34.1)	44.3d (-53.3)	156.6b (+27.8)	234.6a (+49.7)	236.6a (+80.9)	340.3a (+58.1)
Trichoderma harzianum	1693.0d (-39.5)	43.6d (-54.0)	139.6c (+19.0)	156.3b (+24.5)	233.6a (+80.7)	327.6c (+56.5)
Pseudomonas fluorescens race 1	1892.0b (-32.4)	41.3f (-56.4)	136.6c (+17.3)	113.0e (-4.2)	105.6c (+57.4)	326.6c (+56.4)
Pseudomonas fluorescens race 2	1836.6c (-34.3)	50.6b (-46.6)	128.2d (+11.8)	124.0c (+4.8)	119.6b (+62.3)	335.3ab (+57.5)

*Means in each separated column followed by the same letter(s) are not significantly different at P ≤0.05 according to Duncan's multiple range test.*values between parentheses refer to percentages of the reduction (-) and the increasing (+) % according to the equations (1 and 2).

Table 6: Effect of biocontrol agents on nematodes associated with mandarin trees after four weeks of application.

Treatments	Nematode numbers in 250g soil The first season					
	T. semipenetrans	*Xiphinema* spp.	*Tylenchus* spp.	*Dorylaimus* spp.	*Mononchus* spp.	Free-living nematodes
Check (Untreated)	2556.0a (0)	92.0a (0)	95.6e (0)	103.3f (0)	62.0e (0)	202.3d (0)
Nematicide	690.0f (-73.0)	14.0f (-84.7)	31.3f (-67.2)	25.6g (-75.1)	15.0f (-75.8)	29.3e (-85.5)
Marine red yeast (*Rhodosporidium paludigenum*)	1034.6e (-59.5)	28.3c (-69.2)	202.3a (+52.7)	189.0d (+45.3)	153.3d (+59.5)	513.3c (+60.5)
Trichoderma asperellum	1082.6d (-57.6)	25.0d (-72.8)	189.6b (+49.5)	289.3a (+64.2)	393.0a (+84.2)	601.6a (+66.3)
Trichoderma harzianum	1209.0c (-52.7)	24.3ed (-73.5)	174.6c (+45.2)	220.3b (+53.1)	390.3a (+84.1)	590.6b (+65.7)
Pseudomonas fluorescens race 1	1256.6b (-50.8)	22.3ed (-75.7)	172.3c (+44.4)	182.3ed (+43.3)	177.6c (+65.1)	590.3b (+65.7)
Pseudomonas fluorescens race 2	1213.3c (-52.5)	30.6b (-66.6)	164.6d (+41.9)	192.3c (+46.2)	190.0b (+67.3)	597.6a (+66.1)
The second season						
Check (Untreated)	3190.0a (0)	121.3a (0)	115.6f (0)	105.0f (0)	65.0e (0)	204.6e (0)
Nematicide	834.3f (-73.8)	17.0g (-85.9)	37.6g (-67.4)	31.0g (-70.4)	18.3f (-71.8)	35.6f (-82.5)
Marine red yeast (*Rhodosporidium paludigenum*)	1251.0e (-60.7)	34.3c (-71.7)	244.6a (+52.7)	228.6cd (+54.0)	185.3d (+64.9)	620.6d (+67.0)
Trichoderma asperellum	1308.6d (-58.9)	30.3d (-75.0)	229.3b (+49.5)	349.6a (+69.9)	475.0a (+86.3)	727.3a (+71.8)
Trichoderma harzianum	1461.6c (-54.1)	29.3de (-75.8)	211.3c (+45.2)	266.3b (+60.5)	472.0a (+86.2)	714.6bc (+71.3)
Pseudomonas fluorescens race 1	1519.3b (-52.3)	27.0f (-77.7)	208.3cd (+44.4)	220.3e (+52.3)	214.6bc (+69.7)	713.6bc (+71.3)
Pseudomonas fluorescens race 2	1466.3c (-54.0)	37.0b (-69.4)	200.0e (+42.1)	232.3c (+54.8)	229.6b (+71.7)	722.6b (+71.6)

*Means in each separated column followed by the same letter(s) are not significantly different at P ≤0.05 according to Duncan's multiple range test.*values between parentheses refer to percentages of the reduction (-) and the increasing (+) % according to the equations (1 and 2).

were obtained in Tasp, which recorded 84.2 and 66.7% in contrast, the lowest increase was 59.5 and 60.5% in MredY for the same species sequentially. In the second season, results look like they are going in the same direction; the nematicide was superior in its effect on citrus, and dagger nematodes it reduced by 73.8 and 85.9%, respectively. No significant differences were recorded between Thar and Pfr2 in their effect on citrus nematode. MredY was the superior effective microbe that reduced citrus nematode by 60.7%, while Pfr1 was the most effective in reducing dagger nematode by 77.7%. NTNs increased dramatically after four weeks of application; *Mononchus* spp. increased by 86.3% in Tasp, while free-living nematodes increased by 71.8% in the same treatment. The numbers in *Tylenchus* spp. and *Dorylaimus* spp. increased, but to a lesser extent, recorded 52.7% in MredY for the first species and 69.9% in Tasp for the second species. The results in this paper indicated that MredY was the most effective microbe on *T. semipenetrans* while Pfr1 was the lowest effective compared to the nematicide oxamyl after four weeks of application. On the other hand, Thar was the top microbe in its effect on *Xiphinema* spp., and Pfr2 was the lowest. These differences may be due to the variance between each nematode species in its behavior, the difference in the mode of action of these microbes (Köhl et al., 2019; Zhang et al., 2020), and the increase in other nematode species that could compete with them (Abd-Elgawad and Askary, 2020; Mateille et al., 2020). These results may confirm the importance of these microbes in preserving the balance of the nematode community in citrus trees and decreasing PPNs.

Furthermore, it opens up a field in the future to study the effect of mixing these microbes, which may increase their effectiveness in controlling the PPNs. Many authors refer to the role of the microorganisms in controlling PPNs which agree with the obtained results; e.g., Mhatrea et al. (2019) described the role of plant growth promoting rhizobacteria (PGPR), including *Pseudomonads* spp. against citrus PPNs and they decided that all species had a lethal effect on the determining nematode species. Kaur et al. (2020) tested the effect of different biopesticides, including *T. harzianum, T. viride*, and *P. fluorescens,* on *M. incognita, Helicotylenchus* spp., and *X. basiri* populations. They found all products reduced PPN numbers. Asghar et al. (2021) isolated many antagonistic bacteria and fungi from *T. semipenetrans* infesting citrus trees in Punjab, Pakistan. They identified the bacteria species *P. fluorescens, P. putida, Bacillus cereus*, and *B. subtilis,* while the fungi species were *T. harzianum, T. viride, T. koningii,* and *T. atroviride*.

No previous reviews were obtained about the direct effect of marine red yeast on PPNs. The mode of action of this microbe is not precisely defined against PPNs. However, in Egypt, Shawky et al. (2006) tested the efficacy of fungi *T. harzianum* and three yeast isolates of *Saccharomyces* spp. against *M. javanica* under lab, greenhouse, and field conditions. The tested microbes reduced the nematode numbers under all conditions, and *Saccharomyces cerevisiae* was the highest effective isolate cell on root-knot nematode juvenile mortality. El-Qurashi et al. (2019) studied the efficacy of some biocontrol agents against *M. javanica* infesting pomegranate, including the yeast *Pichia guilliermondii*. They indicated that this yeast could reduce *M. javanica* J2 by 25.24%, and they decided that this microbe seemed to be a promising biocontrol agent against root-knot nematodes.

The lethal effect of the marine red yeast may be due to the produce indole-3-acetic acid (IAA); this auxin promotes plant growth and enhances natural defense against nematodes (Limtong et al., 2014; Nutaratat et al., 2015). Farahat et al. (2018) suggested that IAA reduces *Rotylenchulus reniformis* (semi-endo parasitic nematodes similar to *T. semipenetrans*) under field conditions when applied as a foliar spray. Furthermore, this auxin directly affected PPNs

and could be used as a biocontrol agent (Ruanpanun et al., 2010).

Regarding the effect of the tested microbes on NTNs, an unexpected increase in NTNs number has been recorded. The reason for this increase is unclear. Most of these species feed facultatively on yeast, bacteria, and fungi (Hodda, 2022) except *Mononchus* spp., which feeds on the other nematode species (Bastian, 1865). However, the beneficial biological role of these NTNs in the soil is already known (Neher, 2001; Khan and Kim, 2007; Qiaofang et al., 2020; Khanum et al., 2021). Many factors can control the NTN numbers; the most important is soil acidity (pH). Soils with acidic pH increase non-parasitic nematode reproduction and enhance soil microbes (Matute et al., 2012; Hossain et al., 2016; Nisa et al., 2021). Furthermore, most microorganisms can accelerate the soil organic matter decomposition process, which makes it more suitable for NTNs (Jiajia et al., 2022). Many authors revealed that soil organic matter decomposition could increase predaceous, microbivorous, and free-living nematodes (Briar et al., 2007; Yadav et al., 2018). Timper et al. (2021) suggested that intercropping winter cover crops with cotton plants could reduce the early infestation with *M. incognita* for this economic plant by enhancing the numbers of free-living, carnivorous, and omnivorous nematodes. They found a negative correlation between the number of *Mononchus, Tylenchus,* and *Dorylaimus* and *M. incognita* J2 numbers. These results support the correlation hypothesis between the increase in NTNs number and decreasing in PPNs**.**

CONCLUSION

It could be concluded that the *R. paludigenum* had a potential suppressive role on PPNs, especially citrus nematodes, and could be used as a biocontrol agent. Moreover, the role of this microbe had not been studied previously against PPNs. On the contrary, this effect was different on the NTNs, which explains the role of this microbe in maintaining the balance in the soil nematode community in citrus orchards. On the other hand, the obtained results confirmed the role of *P. fluorescens* and *Trichoderma* spp. (tested species) on PPNs, which was studied extensively previously, including limited data on *T. semipenetrans* and other NTNs. The importance of this study is represented by focusing on the role of the tested microbes as a biocontrol agent against PPNs in citrus trees to avoid the environmental hazards of using chemical nematicides and increase other beneficial nematode species in the soil.

DECLARATION

The authors declare that they do not have any actual or potential conflict of interest.

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الملخص العربي

تقييم فاعلية بعض الميكروبات ضد مجتمع النيماتودا (الأنواع المستهدفة وغير المستهدفة) المصاحبة لأشجار الموالح في مصر

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تم تقييم فاعلية خمس ميكروبات على مجتمع النيماتودا المصاحب لأشجار الموالح تحت الظروف الحقلية في مصر. كانت هذه الميكروبات فطري *Trichoderma asperellum* و*T. harzianum* وسلالتين من البكتريا *Pseudomonas fluorescens* والخميرة *Rhodosporidium paludigenum* والميكروب الأخير لم يدرس سابقا ضد النيماتودا المتطفلة على النبات (PPNs). خفضت كل الميكروبات المختبرة من تعداد النيماتودا المتطفلة علي النبات تحت الظروف الحقلية وازداد الانخفاض بمرور الوقت بعد المعاملة و كانت الخميرة *R. paludigenum* الأكثر تأثيرا علي نيماتودا الموالح *Tylenchulus semipenetrans* فقد بلغت نسبة الانخفاض في تعداد الطور اليرقي الثاني (J2) ٥٩,٥ و ٦٠,٧% بعد أربعة أسابيع من المعاملة خلال الموسم الأول و الثاني من الدراسة علي التوالي ، في حين كانت السلالة الاولي من *P. fluorescens* الأكثر كفاءة ضد النيماتودا الخنجرية *Xiphinema* spp. حيث خفضت التعداد بنسبة ٧٥,٧ و ٧٧,٧% خلال نفس الفترة في موسمي الدراسة علي التوالي. علي النقيض من ذلك لم يحدث أي تأثير مثبط للميكروبات المختبرة على أنواع النيماتودا غير المستهدفة (NTNs) وزادت اعدادها بعد المعاملة. عل سبيل المثال زادت اعداد نيماتودا *Tylenchus* بنسبة ٥٢,٧% بعد أربعة أسابيع من المعاملة بالخميرة *R. paludigenum* وذلك في الموسم الثاني من الدراسة في حين زادت اعداد النيماتودا *Dorylaimus* و*Mononchus* وfree-living بنسب ٦٩,٩ و٨٦,٣ و٧١,٨% بعد المعاملة بفطر *T. asperellum* على التوالي بعد أربعة أسابيع من المعاملة. من جانب اخر تم تقييم هذه الميكروبات ضد نيماتودا *T. semipenetrans* تحت ظروف المعمل لتأكيد التأثير على الطور اليرقي الثاني والبيض حيث كانت النتائج المتحصل عليها في تناغم مع نتائج الدراسة الحقلية. تهدف هذه الدراسة لتقييم تأثير الميكروبات المختبرة على النيماتودا النافعة المصاحبة لأشجار الموالح مع التأكيد علي دور هذه الميكروبات كعامل مكافحة حيوية للنيماتودا المتطفلة علي النبات و التركيز علي دور الخميرة *R. paludigenum* كعامل مكافحة حيوية واعد لم يختبر من قبل بشكل وافي علي مجتمع النيماتودا.