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Evidence of inhibitory effect of *Pseudomonas fluorescens* CHA0 and aqueous extracts on tomato plants infected with *Meloidogyne javanica* (Tylenchida: Heteroderidae)

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Abstract

Effects of *Pseudomonas fluorescens* L. (jimsonweed) (*Pf*) isolate and the two plant extracts, *Datura stramonium* and *Myrtus communis*, were investigated on hatching and juvenile (J_2s) mortality of *Meloidogyne javanica* (Tylenchida: Heteroderidae) under laboratory conditions. After determining the values of LC_{30}, LC_{50}, and LC_{70} of each extract, four leaf stage seedlings of tomato were treated by 20 ml of *Pf* suspension at a concentration of 10^8 CFU/ml, using a soil drenching method. After 1 week, the tested plants were inoculated by 4000 eggs and (J_2s) of *M. javanica* and simultaneously were treated by 100 ml of the selected concentrations of *D. stramonium* (1.1, 1.4, and 1.8%) and *M. communis* (1.8, 3 and 5.2%), as soil drench. Results showed that a combination of *Pf* and the leaf extract, *D. stramonium* at the rate of 1.8% or *M. communis* at the rate of 5.2%, respectively, reduced the number of eggs per root system and the reproduction factor by 68 and 45%, the number of galls by 64 and 33%, and the number of egg masses by 65 and 43%, than the control. In conclusion, combination of *Pf* and *D. stramonium* at the rate of 1.8% or *M. communis* at the rate of 5.2% can significantly reduce the damage of *M. javanica* on tomato, under greenhouse conditions.

Keywords: *Pseudomonas fluorescens*, Plant extracts, *Meloidogyne javanica*, Biological control, Tomato

Background

Root-knot nematodes (*Meloidogyne* spp.) are among the most dangerous plant parasites (Jones et al. 2013) which cause a loss of 8.8 to 14.6% to agricultural products annually (Nicol et al. 2011). The nematodes have a short life cycle, a wide host range, and a high reproduction rate; therefore, their management is very difficult (Trudqill and Blok 2001). Besides, the application of chemical nematicides is harmful to the environment; their use also is not economically feasible. Therefore, due to their health consequences for human beings, application of chemical pesticides is limited and the researchers are searching for some safe and environmentally friendly methods which are based on economic and environmental issues. Some examples of such approaches are using plant extracts and herbal products like root exudates, herbal meal, and medicinal plant wastes (Marahatta et al. 2012). Biological control agents (Lamovsek et al. 2013; Silva et al. 2017) and their products (Radwan et al. 2012) are used in integrated pest management program.

Among the bio agents, plant growth-promoting rhizobacteria (PGPR)—such as *Pseudomonas fluorescens* CHA0—has a high efficiency in controlling plant pathogens like root-knot nematodes (Tavakol Norabadi et al. 2014). The outstanding feature of *P. fluorescens* is its high solubilization capacity of soil phosphorous (Galindo et al. 2018). Results showed that a mixture of *P. fluorescens* and *Azospirillum brasilense* had a positive influence on the yield of three potato varieties (Trdan et al. 2018).

In the last seven decades, plant extracts and other phytochemicals were surveyed for their effects on plant-parasitic nematodes. Aqueous extracts of different parts of Neem, Chinaberry, and marigold were successfully

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used against the root-knot nematodes (Siddiqui and Shakeel 2007). Based on the chemical analysis of plant tissues from some plants, some chemical compounds have been detected. Marigold with alpha-terthienyl (Hethelyi et al. 1986) and Neem with azadirachtin, nimbin, salannin, nimbidin, and thionemone (Ferraz and de Freitas 2004) showed good effects on root-knot nematodes.

Considering the advantages of biological control, this study aimed to find out suitable aqueous extracts of some plants on juvenile mortality and hatching of *M. javanica* under laboratory conditions. The other target was to evaluate the efficiency of the combination of plant extracts and *P. fluorescens* CHA0 on reducing nematode damage in tomatoes greenhouse.

Materials and methods

Effects of different aqueous extract concentrations of *Datura stramonium* L. (jimsonweed), *Myrtus communis* L. (myrtle), *Fumaria officinalis* L., and *Vitex agnus-castus* L. (Chaste tree) on hatching and juvenile (J_2s) mortality of *M. javanica* were analyzed under laboratory conditions. After identifying the suitable plant extracts, the necessary concentrations to cause 30, 50, and 70% J_2s mortality were used in combination with an isolate of *P. fluorescens* CHA0 on tomato plants.

Preparation of root-knot nematodes culture

The roots of nematode-infected tomatoes were collected from greenhouses at Boyer-Ahmad County, Iran, and a single egg mass of the root-knot nematode, *M. javanica*, was cultured on tomato seedlings (cv. Early-Urbana) in the greenhouse. The root-knot nematodes species were identified based on the study of perennial pattern, as described by Taylor and Netscher (1974). In order to provide the suspension of nematode eggs, the method of Hussey and Barker (1973) was used. By storing the egg suspension in incubator adjusted to 27 °C, J_2s were hatched and were collected over a period of 4 days (Baghaee Ravari and Mahdikhani Moghaddam 2015).

Preparation of plant extracts

The aerial parts of the plants *D. stramonium*, *M. communis*, *F. officinalis*, and *V. agnus-castus* were collected from Boyer-Ahmad County, Iran, dried in shade and finely grinded using an electric grinder and a stock solution (10% w/v) was prepared (Ferris and Zheng 1999).

Preparation of bacterial isolate

The isolate of *P. fluorescens* CHA0 was obtained from the Department of Plant Protection, Faculty of Agriculture, Tehran University, Iran. To obtain a pure and fresh bacterial culture, bacterial suspension was grown on Nutrient Agar (NA) culture. The grown bacteria were harvested and mixed up with distilled water, and then the concentration was adjusted to 10^8 CFU/ml.

Laboratory assay

Two laboratory experiments were conducted to examine the inhibitory effect of plant extracts and bacterial suspension on J_2s hatching and mortality in *M. javanica*. One milliliter of the egg suspension containing 100 ± 10 of nematode eggs was poured into the Petri dishes (with a diameter of 8 cm, then, 9 ml) of bacterial suspension with the concentration of 10^8 CFU/ml, or aqueous plant extracts at the rates of 0.5, 1, 2, 4, 6 and 8% (w/v) were added and then they were kept under controlled conditions at 27 °C. After two periods of 72 and 120 h, the hatched juveniles were counted, using a stereo microscope (Gökhan and Sevilhan 2014). In another experiment, the J_2s mortality was investigated. Nine milliliters of bacterial suspension with the concentration of 10^8 CFU/ml, or aqueous plant extracts at the rates of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, and 8% were added to 1 ml of nematode suspension containing 100 ± 10 J_2s of *M. javanica* in Petri dishes and kept at 27 °C, under controlled conditions. Dead J_2s were counted with the help of a stereo microscope (Dourado et al. 2013). The experiments were carried out in completely randomized designs with four replications. The lethal mortality values (LC_{30}, LC_{50}, and LC_{70}) necessary to cause 30, 50, and 70% J_2s mortality, were subjected to probit analysis in order to find the suitable concentrations.

Greenhouse experiment

Two greenhouse experiments were conducted at Boyer-Ahmad County, Iran, in 2017 and 2018. Different lethal concentrations (LC_{30}, LC_{50}, and LC_{70}) of *D. stramonium* and *M. communis* were chosen for greenhouse test. Seeds of susceptible tomato (cv. Early-Urbana) were sown in plastic pots with 1000 g of steam sterilized soil mixture of farm soil (sandy loam soil with electrical conductivity (EC) = 0.671 dS m^{-1}, pH = 7.45, contains 76% calcium carbonate, 52.9 mg/kg phosphorus, 0.170 mg/kg of organic matter and 0.0987 mg/kg of organic carbon), cow manure and sand with a ratio of 1:1:2, respectively. The pots were kept under controlled conditions in the greenhouse with 16:8 h light to dark photoperiod and 27 ± 5 °C. At four leaf stage, each one of the tomato seedlings was treated by 20 ml of a suspension of 10^8 CFU/ml of *P. fluorescens* CHA0 as soil drench. After 7 days, treated seedlings were inoculated simultaneously by 4000 eggs and J_2s of *M. javanica* and soil-drenched with 100 ml/pot of selected concentrations of *D. stramonium*, viz. 1.1, 1.4, and 1.8% (w/v) and *M. communis* viz. 1.8, 3 and 5.2% (w/v). Sixty days after nematode inoculation, plants were harvested and plant

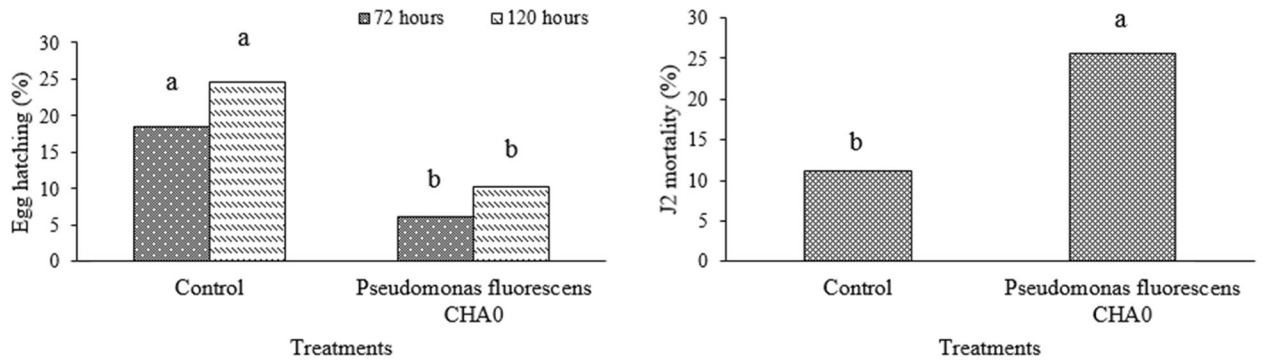

Fig. 1 Mean egg hatching and mortality (%) of *Meloidogyne javanica* juveniles exposed to *Pseudomonas fluorescens* CHA0 at a concentration of 10^8 CFU/ml. Means are average of four replications. Means in a column followed by the same letter(s) are not different according to LSDs ($p \leq 0.01$)

shoot length as well as the fresh, dry weight of shoot, and fresh weight of root was recorded. Number of eggs, galls, and egg masses per root system and number of J_2s per pot were counted, and the reproductive factor of the nematode was calculated as described by Sasser and Taylor (1978). Approximately 2 months after the first trial of the experiment, for the same procedure was done as the second trial of the experiment. The experiments were carried out in a completely randomized design test with five replications.

Statistical analysis

For the greenhouse experiment, data of plant growth parameters were subjected to a 4 × 2 × 2 (plant extracts × bacterial isolate × nematode) factorial analysis of variance (ANOVA) and the data of nematode population indices were subjected to a 4 × 2 (plant extracts × bacterial isolate) factorial analysis of variance (ANOVA) in a completely randomized design, using SAS statistical software (SAS Institute, Cary, NC). For assays of the inhibition of hatching and J_2s mortality, data were subjected to one-way ANOVA. To normalize the data sets, prior to ANOVA analysis, expressed data as percentages were transformed to arcsin values (ArcSin√X) and only untransformed arithmetic means were presented. Where the *F*-test showed significance difference at $p < 0.01$, treatment means were compared using least significant differences (LSDs).

Results and discussions

Laboratory assay

Application of *P. fluorescens* CHA0 significantly reduced the percentage of hatching of *M. javanica* and increased its J_2s mortality than those treatments, which had no bacteria (Fig. 1). The results of *D. stramonium* and *M. communis* extracts on hatching (Fig. 2) and J_{2s} mortality (Fig. 3) indicated that the increase in plant extract concentrations and numbers of hatched eggs decreased, while the numbers of dead J_2s increased. The effects of *V. agnuscastus* and *F. officinalis* extracts were low; therefore, they were not chosen for greenhouse experiments. Toxicity lines were established for both of *D. stramonium* and *M. communis*, then LC_{30}, LC_{50}, LC_{70}, and LC_{90} were calculated (Table 1). The inhibitory effects of the plant extracts against the nematodes may be related to the presence of some metabolites in the plant. These chemicals can either affect the growth of J_2s or kill them inside the egg or can dissolve the egg masses of root-

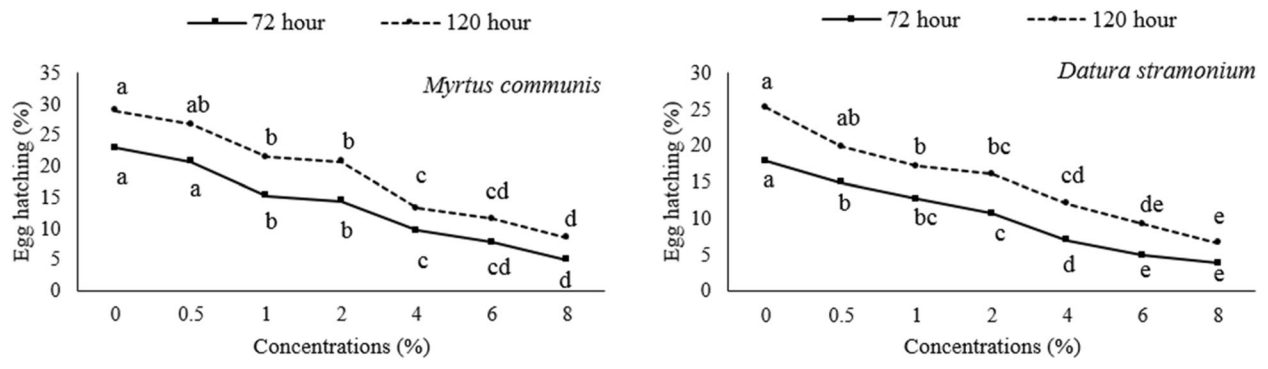

Fig. 2 Mean egg hatching of *Meloidogyne javanica* 72 and 120 h after different concentrations of *Datura stramonium* and *Myrtus communis* treatments. Means are average of four replications. Means followed by the same letter(s) are not different according to LSDs ($p \leq 0.01$)

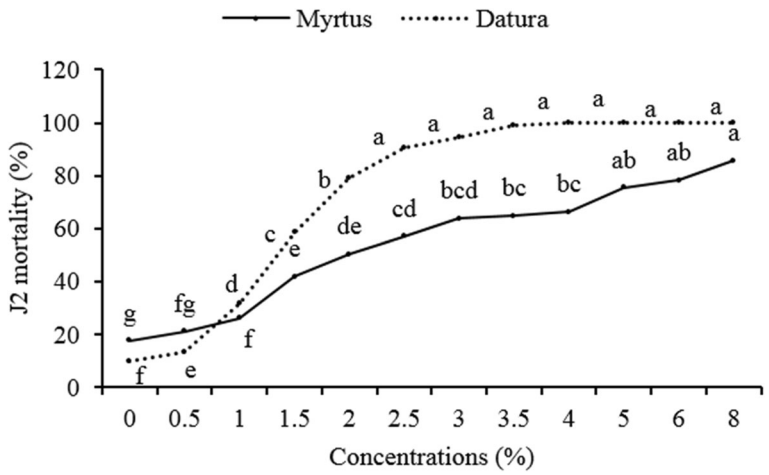

Fig. 3 Mean mortality (%) of *Meloidogyne javanica* juveniles exposed to different concentrations of *Datura stramonium* and *Myrtus communis* extracts. Means are average of four replications. Means followed by the same letter(s) are not different according to LSDs ($p \leq 0.01$)

knot nematodes (Adegbite and Adesiyan 2005). Some plant extracts may affect the behavior of nematodes, such as host finding capabilities and ultimately killing them. This type of response of juveniles and nematode eggs to plant extracts can be due to the differences in the type of plant metabolites (Zuckerman and Esnard 1994). Because of the variations which exist in antimicrobial compounds of plant extracts and essential oils, there are different mechanisms for their activities. Considering the combined activity, these compounds destroy the cell wall membrane and increase cellular permeability and ionic leakage. Following the breakdown of phospholipid molecules of cell wall, mitochondria, and membrane proteins, as well as decomposition of cytoplasm, cells are severely damaged and will die (Burt 2004).

Greenhouse experiment

Plants treated with the extract of *M. communis* at the rate of 5.2% (w/v) had the longest shoot and the highest shoot fresh weight. Non-significant differences were observed between these treatments and nematode non-inoculated plants, which were treated by plant extract at the rate of 3% (w/v) in the case of shoot length. The highest shoot dry weight and root fresh weight were observed in nematode non-inoculated plants that treated with plant extract at the rate of 5.2% (w/v) along with bacterial incubation. The shoot dry weights had non-significant difference with the non-inoculated plants and with myrtle extract at the rate of 5.2% (w/v) (Table 2). The number of eggs, galls, and egg masses per root system and the reproductive factor were significantly reduced in the bacterial-treated and non-treated tomatoes receiving 5.2% (w/v) myrtle extract. They had significant difference than other treatments, except bacterial-treated and non-treated plants containing 3% (w/v) myrtle extract. The lowest numbers of J_2s were observed in the soil of bacterial-treated and non-treated tomatoes applied with 5.2% (w/v) myrtle extract. It had non-significant difference with bacterial-treated plants with 3% myrtle extract (Table 3).

Table 1 Estimated values of lethal concentrations (%) (LC_{30}, LC_{50}, and LC_{70}) of *Meloidogyne javanica* juveniles 48-h post-exposure to different concentrations of *Myrtus communis* and *Datura stramonium* extract

Plant species	LC_{30}	LC_{50}	LC_{70}	R^2	Regression equations
Myrtus communis	1.8%	3%	5.2%	0.97	$Y = 1.7478x + 4.1984$
Datura stramonium	1.1%	1.4%	1.8%	0.98	$Y = 4.9057x + 3.7725$

The results of application of *D. stramonium* extract indicated that the longest shoots were observed in nematode non-inoculated plants, when treated with the plant extract at the rate of 1.8% (w/v) with and without *P. fluorescens* CHA0 incubation. They indicated non-significant difference with nematode inoculated and bacterial-treated plants with plant extract at the rate of 1.8% (w/v) and with nematode non-inoculated plants treated with plant extract at the rate of 1.4% (w/v) without *P. fluorescens* CHA0 incubation. The highest shoot fresh weight was observed in healthy plants that were treated with 1.8% (w/v) plant extract, with or without *P. fluorescens* CHA0 incubation. They indicated non-significant difference with bacterial-treated nematode-infected plants along with plant extract at the rate of 1.8% (w/v) and also with treated healthy plants with plant extract at the rate of 1.4% (w/v) with or without bacterial incubation. The highest shoot dry weights were in healthy treated plants with 1.8% (w/v) concentration with and without bacterial incubation. They had non-significant difference with bacterial-treated and nematode-infected plants that treated

Table 2 Mean plant growth indices of tomato that was either inoculated or non-inoculated with *Meloidogyne javanica* along with *Pseudomonas fluorescens* CHA0 and different concentrations of *Myrtus communis* treated in trials I and II

Concentrations (%)	Bacteria	Nematode	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)
0	Non-treated	Non-inoculated	42.30 ± 1.56 ef	39.70 ± 1.69 hij	4.93 ± 0.32 fgh	4.70 ± 0.43 h
		Inoculated	34.95 ± 1.72 f	37.13 ± 1.41 j	4.18 ± 0.25 h	4.75 ± 0.13 h
	Treated	Non-inoculated	47.35 ± 1.17 de	44.85 ± 1.38 f-j	5.29 ± 0.1 e-h	5.63 ± 0.29 gh
		Inoculated	41.55 ± 1.71 ef	38.93 ± 1.44 ij	4.81 ± 0.15 fgh	5.63 ± 0.35 gh
1.8	Non-treated	Non-inoculated	46.83 ± 2.25 de	41.35 ± 2.4 g-j	6.37 ± 0.37 def	6.18 ± 0.23 fgh
		Inoculated	47.68 ± 0.8 de	41.68 ± 0.86 g-j	4.68 ± 0.34 gh	5.65 ± 0.35 gh
	Treated	Non-inoculated	53.60 ± 3.59 bcd	47.35 ± 2.47 d-h	6.78 ± 0.49 cde	7.36 ± 0.24 d-g
		Inoculated	51.43 ± 2.53 cd	46.13 ± 1.62 e-i	5.20 ± 0.48 e-h	5.90 ± 0.45 fgh
3	Non-treated	Non-inoculated	57.68 ± 2.54 abc	53.43 ± 2.44 b-d	7.88 ± 0.43 cd	7.45 ± 0.32 c-f
		Inoculated	52.43 ± 2.92 bcd	49.40 ± 1.35 c-g	6.00 ± 0.30 efg	6.88 ± 0.28 efg
	Treated	Non-inoculated	59.80 ± 2.06 abc	58.73 ± 2.77 ab	8.28 ± 0.38 bc	9.75 ± 0.76 b
		Inoculated	56.95 ± 2.11 bc	52.45 ± 1.00 b-f	6.43 ± 0.45 def	7.35 ± 0.45 d-g
5.2	Non-treated	Non-inoculated	57.98 ± 3.6 abc	57.78 ± 2.68 ab	9.53 ± 0.35 ab	9.00 ± 0.57 bcd
		Inoculated	58.10 ± 1.11 abc	54.78 ± 2.36 a-d	8.17 ± 0.37 bc	8.38 ± 0.28 b-e
	Treated	Non-inoculated	65.55 ± 1.76 a	62.68 ± 2.53 a	10.05 ± 0.51 a	11.43 ± 0.67 a
		Inoculated	60.48 ± 3.73 ab	57.15 ± 1.46 abc	8.13 ± 0.56 bc	9.13 ± 0.35 bc

Values in the same column followed by different letter(s) are significantly different according to LSDs ($p \leq 0.01$). Each treatment had ten replications (five replications in two successive experiments). Values are means ± standard error

with plant extract at the rate of 1.8% (w/v). The highest root fresh weights were observed in healthy bacterial-treated plants with 1.8% (w/v) of plant extract (Table 4). Numbers of eggs per root system and the reproductive factor of nematode in bacterial-treated and non-treated tomato roots, receiving 1.8% (w/v) of *D. stramonium* extract, were significantly reduced than other treatments. The lowest numbers of J_2s in soil and egg masses per root system were observed in the root of bacterial-treated and non-treated plants with 1.8% (w/v) plant extract. There were non-significant differences with bacterial-treated plants receiving 1.4% (w/v) plant extract. The lowest numbers of galls per root system were observed on the roots of bacterial-treated plants that were treated with 1.8% (w/v) plant extract. There were non-significant differences between those and bacterial non-treated plants with 1.8% (w/v) plant extract (Table 5).

The positive effects of *D. stramonium* and *M. communis* extracts in reducing the damages caused by root-knot nematodes have been shown in several studies. The powder of *D. stramonium* at the rates of 75 g/kg of soil caused an improvement in okra growth indices and decreased the *M. incognita* infections (Hussain et al. 2011). It was indicated that *D. stramonium* extract contains large quantities of saponin and flavenoid and small quantities of tannin and alkaloid so it caused mortality

Table 3 Mean numbers of galls, egg masses, and eggs per root, second-stage juveniles (J_2s) per pot, and reproductive factor (RF) of *Meloidogyne javanica* after nematode inoculation along with *Pseudomonas fluorescens* CHA0 incubation of tomato plants and different concentrations of *Myrtus communis* treated in trials I and II

Concentrations (%)	Bacteria	Number of eggs/root	Number of galls/root	Number of egg masses/root	Number of J2 s/pot	Reproduction factor
0	Non-treated	79173 ± 3409 a	891 ± 21.79 a	588 ± 23.07 a	905 ± 48.45 a	20.16 ± 0.86 a
	Treated	65374 ± 3151 b	896 ± 41.30 a	583 ± 27.22 a	862 ± 44.91 a	16.70 ± 0.80 b
1.8	Non-treated	62209 ± 3382 bc	820 ± 36.37 ab	501 ± 28.00 ab	790 ± 41.349 ab	15.87 ± 0.86 bc
	Treated	61673 ± 3539 bc	813 ± 42.11 ab	510.5 ± 26.62 ab	798 ± 51.01 ab	15.74 ± 0.90 bc
3	Non-treated	49533 ± 2189 cd	731 ± 19.54 bc	420.5 ± 36.502 bc	704 ± 24.84 bc	12.66 ± 0.56 cd
	Treated	47813 ± 2988 d	686 ± 38.23 bc	393.5 ± 31.50 bc	604 ± 27.01 cd	12.20 ± 0.76 d
5.2	Non-treated	45239 ± 3484 d	658 ± 26.46 c	384.25 ± 21.43 c	519 ± 22.12 d	11.53 ± 0.88 d
	Treated	43637 ± 3268 d	593 ± 26.87 c	332.25 ± 23.43 c	487 ± 25.56 d	11.11 ± 0.82 d

Values in the same column followed by different letter(s) are significantly different according to LSDs ($p \leq 0.01$). Each treatment had ten replications (five replications in two successive experiments). Values are means ± standard error

Table 4 Mean plant growth indices of tomato, inoculated or non-inoculated with *Meloidogyne javanica* along with *Pseudomonas fluorescens* CHA0 and different concentrations of *Datura stramonium* treated in trials I and II

Concentrations (%)	Bacteria	Nematode	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)
0	Non-treated	Non-inoculated	42.3 ± 1. f	39.7 ± 1.69 e	4.925 ± 0.31 g	4.7 ± 0.43 g
		Inoculated	34.95 ± 1.72 g	37.12 ± 1.41 e	4.175 ± 0.24 g	4.75 ± 0.13 g
	Treated	Non-inoculated	47.35 ± 1.16 ef	44.85 ± 1.3 de	5.285 ± 0.39 fg	5.67 ± 0.27 fg
		Inoculated	41.55 ± 1.70 f	38.92 ± 1.43 e	4.81 ± 0.15 g	5.62 ± 0.35 fg
1.1	Non-treated	Non-inoculated	57.55 ± 1.24 c	52.15 ± 2.19 cd	7.47 ± 0.37 cde	6.65 ± 0.4 efg
		Inoculated	49.52 ± 1.62 de	53.67 ± 1.83 cd	5.85 ± 0.66 efg	7.12 ± 0.26 def
	Treated	Non-inoculated	55.15 ± 1.17 cd	58.025 ± 2.504 bc	7.8 ± 0.38 cde	9 ± 0.71 bcd
		Inoculated	52.52 ± 1.32 cde	50.875 ± 0.80 cd	7.125 ± 0.60 def	7.87 ± 0.51 de
1.4	Non-treated	Non-inoculated	66.1 ± 1.33 a	62.95 ± 3.84 ab	9.5 ± 0.47 bc	7.85 ± 0.37 de
		Inoculated	52.87 ± 1.75 de	57.05 ± 2.13 bc	8.575 ± 0.31 cd	10.24 ± 0.52 bc
	Treated	Non-inoculated	59.25 ± 2.22 bc	66.925 ± 2.46 a	9 ± 0.44 cd	10.75 ± 0.98 b
		Inoculated	57.95 ± 2.20 bc	55.45 ± 1.45 bc	8.2 ± 0.40 cd	9.05 ± 0.23 bcd
1.8	Non-treated	Non-inoculated	67.17 ± 2.792 a	63.425 ± 2.62 ab	11.1 ± 0.87 ab	8.16 ± 0.38 cde
		Inoculated	58.02 ± 1.50 bc	55.85 ± 2.15 bc	8.07 ± 0.49 cd	10.06 ± 0.52 bc
	Treated	Non-inoculated	70.67 ± 1.72 a	67.95 ± 1.83 a	11.82 ± 0.40 a	13.17 ± 0.60 a
		Inoculated	64.82 ± 1.48 ab	67.57 ± 3.12 a	11.645 ± 0.88 a	10.8 ± 0.72 b

Values in the same column followed by different letter(s) are significantly different according to LSDs ($p \leq 0.01$). Each treatment had ten replications (five replications in two successive experiments). Values are means ± standard error

of J_2s of *M. javanica* in laboratory conditions, and also, it caused a significant decrease in nematode indices and a significant growth improvement in infected melon (Umar and Ngwamdai 2015). Phytochemicals, such as saponins, tannins, flavonoids, alkaloids, phenols, and steroids, cause a significant decrease in reproductive factor as well as the numbers of galls and eggs of *M. incognita*, and they increase the amount of yield and plant growth indices. In a study carried out on ethanolic and aqueous extracts of *D. stramonium*, *D. innoxia*, and *D. tatula*, the ethanolic extracts had more effects on egg hatch inhibition and increase the J_2s mortality in *M. incognito*. Moreover, they reduced nematode infection but were uninfluential to plant growth indices (Babaali et al. 2017). It is also indicated that with an increase in the concentration of aqueous extracts of *D. stramonium*, the J_2s mortality was increased (Sidhu et al. 2017), which is consistent with the results of the present study.

In previous studies, the antibacterial effects of myrtle extracts were analyzed (Mansouri et al. 2001; Amensour et al. 2010). The nematicidal properties of these antibacterial compounds have not been proven so far. In the study of Oka et al. (2012), the effects of aqueous extract and powder of *M. communis* on J_2s mortality in *M.*

Table 5 Mean numbers of galls, egg masses and eggs per root, second-stage juveniles (J_2s) per pot, and reproductive factor (RF) of *Meloidogyne javanica* after nematode inoculation along with *Pseudomonas fluorescens* CHA0 incubation of tomato plants and different concentrations of *Datura stramonium* treated in trials I and II

Concentrations (%)	Bacteria	Number of eggs/root	Number of galls/root	Number of egg masses/root	Number of J2 s/pot	Reproduction factor
0	Non-treated	81894 ± 1949 a	913 ± 24.75 a	706 ± 15.80 a	897.75 ± 29.88 a	20.87 ± 0.49 a
	Treated	52834 ± 3843 b	761 ± 29.88 b	412 ± 25.54 b	529 ± 60.23 b	13.44 ± 0.98 b
1.1	Non-treated	47883 ± 2692 bc	601 ± 26.79 c	414 ± 30.31 b	525.25 ± 34.7b5	12.20 ± 0.65 bc
	Treated	44092 ± 2174 bc	585 ± 31.80 cd	364 ± 25.236 bc	431 ± 29.33 bc	11.22 ± 0.55 bc
1.4	Non-treated	41345 ± 1921 c	487 ± 22.95 de	346 ± 14.60 bc	406 ± 34.77 bc	10.52 ± 0.49 c
	Treated	38519 ± 2030 c	476.5 ± 25 e	318 ± 15.83 cd	356 ± 22.43 cd	9.798 ± 0.50 c
1.8	Non-treated	27559 ± 2452 d	381 ± 21.97 ef	309 ± 16.77 cd	319.75 ± 10.61 cd	7.04 ± 0.61 d
	Treated	26315 ± 2869 d	325 ± 24.14 f	249 ± 20.10 d	255 ± 13.93 d	6.704 ± 0.72 d

Values in the same column followed by different letter(s) are significantly different according to LSDs ($p \leq 0.01$). Each treatment had ten replications (five replications in two successive experiments). Values are means ± standard error

javanica in soil and the number of eggs per root system and gall index were investigated and the results proved the nematicidal activities of myrtle plant.

Induced systemic resistance (ISR) in response to rhizospheric bacteria is one of the mechanisms of resistance against plant pathogenic nematodes, such as root-knot nematodes. Systematic resistance, caused by *P. fluorescens* CHA0, is attributed to the secondary metabolite, namely 2, 4-diacetylphloroglucinol (Siddiqui and Shaukat 2003). This kind of resistance which is caused by *P. fluorescens* CHA0 was reported in other studies as well. Rhizobacterial isolates have different mechanisms in inhibiting the life cycle of plant-parasitic nematodes (Siddiqui and Mahmood 1999). The production of hydrogen cyanide, ammonium, hydrogen sulfide, antibiotic, and volatile fatty acids are other inhibitory mechanisms of *P. fluorescens* CHA0. These toxic metabolites affected the reproductive rate of nematodes, therefore decreasing the number of nematodes (Siddiqui et al. 2006). Furthermore, the antagonistic fluorescent bacterial used against plant-parasitic nematodes, which are compatible with the rhizospheres, has low costs and has no adverse consequences for the environment.

Conclusion

The study indicated that aqueous extracts of *D. stramonium* and *M. communis* in combination with *P. fluorescens* CHA0 had a potential to decrease the reproduction of the root-knot nematode, *M. javanica*, and can improve the plant growth indices of infected tomatoes under greenhouse conditions.

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Authors' contributions

HC and RR conceived and designed research. AM, HC, and MA conducted the experiment. AM and RR studied bacterial experiments. HC analyzed the data. HC and MA wrote the manuscript. All authors read and approved the final manuscript.

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Competing interests

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