Effectiveness of salicylic acid, *Pseudomonas fluorescens* CHA0 and *Trichoderma viride* to control *Meloidogyne incognita* race 2 on different tomato cultivars

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Summary The effects of salicylic acid (SA), *Trichoderma viride* and *Pseudomonas fluorescens* CHA0 were studied on the root-knot nematode *Meloidogyne incognita* race 2 in resistant and susceptible tomato cultivars (Gina VF, Falat CH, Falat 111, Karoon) during 2012–2013. Four-leaf tomato seedlings were used, grown in pots containing 1000 g of sterilized soil; each seedling receiving 20 ml of *T. viride* suspension containing 1×10^6 spores, 30 ml of *P. fluorescens* CHA0 with 10^9 cfu/ml, 5mM of salicylic acid and 2000 second stage nematode juveniles. Parameters relevant to nematode population and plant growth were evaluated. The biocontrol agents and salicylic acid were effective in nematode control in combined and single treatments. High reductions in root galling and egg mass indices were observed with combination of SA and biocontrol agents. The greatest increase in plant growth was obtained when cv. Falat CH was treated with SA followed by *P. fluorescens* CHA0 and *T. viride*. The highest number of galls was recorded in cv. Karoon, followed by cvs. Falat 111, Gina VF and Falat CH. *Pseudomonas fluorescens* CHA0 provoked the highest increase in fresh and dry root weight, fresh and dry shoot weight and plant length in all free nematode treatments. The results indicated that chemical inducer (salicylic acid), in combination with biocontrol agents (*T. viride* and *P. fluorescens* CHA0), stimulated and eventually increased plant growth.

Additional keywords: biocontrol, *Lycopersicon esculentum*, reproduction factor, root-knot nematode

Introduction

Root knot nematodes are one of the most important plant parasites. They have a wide host range including more than 2000 plant species (Gugini *et al.*, 2008). The primary symptom of root-knot nematode infection is the formation of typical root galls on the roots of susceptible host plants. Nutrient and water uptake are substantially reduced because of the damaged root system, resulting in weak and low-yielding plants (Abad *et al.*, 2003). *Meloidogyne* spp. can severely damage important crops including tomato, cucumber and melon in many areas. The nematodes are major pests on tomatoes causing considerable yield losses (Sahebani and Ha-

davi, 2008).

Biological control is a safe alternative to pesticides for phytonematode management, likely to be free from toxic residual effects. There are numerous microbial antagonists of root-knot nematodes and their application results in significant decrease in the nematode populations (Khan *et al.*, 2007). In fact, a wide range of bacteria (Hallmann *et al.*, 2001) and fungal agents (Meyer *et al.*, 2001) have been used to reduce damages of plant parasitic nematodes. *Pseudomonas fluorescens* CHA0 is a root-colonizing biocontrol bacterium that protects several plant species from root diseases caused by soil-borne fungi and root-knot nematodes (Siddiqui and Shaukat, 2002). Some species of *Trichoderma* spp. have widely been used as biocontrol agents against soil-borne plant diseases (Whipps, 2001) and root-knot nematodes (Sharon *et al.*, 2001). Nandy *et al.* (2003) found that salicylic acid decreased gall and egg numbers of *Meloidogyne incognita* on okra and cowpea. Numerous studies have separately inves-

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tigated the impact of biocontrol agents to control root-knot nematodes on susceptible cultivars. However, comprehensive research about the effects of salicylic acid (SA), *Trichoderma* and *Pseudomonas* simultaneously on *M. incognita* race 2 in both susceptible and resistance tomato cultivars has not yet been implemented. The present study examined the effect of a chemical inducer (SA) and two biocontrol agents (*T. viride* and *P. fluorescens* CHA0), when used independently and in combination, in controlling *M. incognita* race 2 on different tomato cultivars.

Materials and Methods

Nematode Inocula

Infected root samples were collected from naturally infested tomato plants in a greenhouse. Roots were rinsed with water and nematodes were extracted from egg masses present on the galled tissue. The identification of the root-knot nematode species was based on the shape of the perineal pattern (Jepson 1987) according to the methods of Taylor and Netscher (1974). The races of root-knot nematode populations were identified on the basis of differential hosts tests (Hartman and Sasser, 1985). A single egg mass was used to establish a population on tomato plants. Eggs were extracted from galled tomato roots using 1.5% NaOCl (Hussey and Barker, 1973), collected on a 25 µm mesh sieve and transferred to a beaker containing distilled water. Hatched juveniles of *M. incognita* were obtained by placing the eggs in sterile distilled water for 5 days at $28 \pm 2^\circ\text{C}$. The activities of juveniles were evaluated under a stereomicroscope.

Biocontrol Agents

Trichoderma viride and *P. fluorescens* CHA0 were a kind offer of the Plant Pathology Department, Faculty of Agriculture, Shahed University. *T. viride* was cultured on potato dextrose agar (PDA) containing 150 mg l⁻¹ streptomycin and 150 mg l⁻¹ of chloramphenicol, and was kept at 25°C. The mycelia and formed conidia were carefully scraped

from the media and suspended in 100 ml of distilled water. Spores were separated from mycelia by sieving through a 50 µm sieve. The spore suspensions were then adjusted to the desired concentration after counting spore density using a haemocytometer (Sahabani and Hadavi, 2008).

The bacterium isolate was stored in sterile distilled water and was cultured in nutrient agar medium. Density of 10⁹ colony forming unit (cfu) per milliliter of water of 24 h bacterium culture was prepared by serial dilution (10⁻¹–10⁻⁹) in 590 nm (Zhang *et al.*, 2002).

Chemical Agent

Salicylic acid (SA) (Merck) was prepared and dissolved in ethanol and water in concentrations of 5 mM. Firstly, the necessary amounts of SA (0.69 g) were dissolved in 100 ml 96% methanol solution. The volume of the solution was brought to one liter with distilled water. Then, the concentration of 5 mM was used and sprayed on the leaves (Zhang *et al.*, 2002).

Plant Cultures

The tolerant tomato cultivars Gina VF, Falat 111 and the susceptible Falat CH and Karoon were used (Gharabadiyan *et al.*, 2012). Seeds were sown in the pots containing 1000 g of sterilized mixture of field soil, leaf compost and sand at the rate of 1:1:2 in 24–27°C under greenhouse conditions. Three-week-old (four-leaf stage) seedlings were used in the experiments. The seedlings were inoculated with *T. viride*, *P. fluorescens* and salicylic acid, separately and jointly. Each seedling received 20 ml of a liquid suspension of *T. viride* containing 1×10⁶ spores, 30 ml of bacterial suspension 10⁹ cfu/ml and lastly 5mM salicylic acid. Four replicates were maintained for each treatment. The biocontrol agents were used by soil drenching, but SA was applied with foliar spray. The next day, 2000 J₂ (two stage juveniles) of root-knot nematode per plant were used for the artificial inoculation. The inoculum was injected into 3 holes approximately 2 cm deep around the stem base.

Forty five days after inoculation, nematode infestations as well as plant growth parameters were assessed. Two control sets were included, namely uninoculated and artificially inoculated plants with nematodes. The uninoculated control did not receive nematodes and biocontrol agents and SA and the inoculated control received only nematodes. The experiment consisted of 12 treatments: 1) non-inoculated (negative control); 2) only nematode (positive control); 3) T (*Trichoderma*); 4) SA; 5) P (*Pseudomonas*); 6) N+T; 7) N+SA; 8) N+P; 9) N+T+SA; 10) N+P+SA; 11) N+P+T; 12) N+P+T+SA. At harvest, roots were removed, washed free of soil and stained by 0.015% Phloxine B for 20 minutes to facilitate egg mass counting. The number of galls, egg masses per plant and length, fresh and dry weight of plants and nematode population in the soil were measured and recorded (Hussey and Janssen, 2002).

During the experiment, tomato plants were maintained in a greenhouse randomizing the position of the blocks and, at the same time, repositioning each plant within a block every week to avoid a block position effect and the factor 'position of the plant within the block'.

Statistical Analysis

The experimental design was factorial randomized complete block with 4 tomato cultivars, 12 treatments and 4 replications per treatment. The experiment was repeated for two years (2012 and 2013) under the same conditions. Statistical analysis proved that there was no statistically significant difference between the two years in recorded data and hence the results of two years were pooled. Comparison of the means was performed by ANOVA and Tukey test ($P \leq 0.01$). According to Oostenbrinks equation [Oostenbrink, 1966], reproduction factor was calculated for each of the treatments based on $R = PF/PI$ formula where PI is the initial population and PF is the final population. The Final Population density (PF) was obtained by counting second stage juvenile (J_{25}) population in the soil. All data was analyzed by SAS

Software Version 9.00.

Results

Detailed observations of ($n=10-20$) female nematodes indicated that the perineal pattern characteristics and differential host tests correspond to *Meloidogyne incognita* race 2.

The data presented in Table 1 revealed that the combined inoculation of *T. viride* and *P. fluorescens* and SA improved the tomato plants' growth characters compared to the inoculated control.

According to Figure 1, the soil application of biocontrol agents and chemical inducer significantly ($P \leq 0.01$) reduced galling and egg mass production compared to the inoculated control. Treatment 12 (soil drenching of biocontrol agents + foliar spray of SA) exhibited the lowest number of galls and egg masses compared to positive control and other treatments (Figure 1, Figure 2). Application of SA was also found to be effective against the nematode but less effective than *T. viride* and *P. fluorescens* (Figure 1). The greatest numbers of galls were recorded in the inoculated control pots of tomato cv. Karoon, followed by cvs. Falat 111, Gina VF and Falat CH. (Figure 1). Egg masses at all treatments were significantly ($P \leq 0.01$) different compared to the control. There were differences between the four tomato cultivars (Figure 2). Reproduction factor of *M. incognita* race 2 on the cultivars is shown in Figure 3.

The results showed that inoculation with the root-knot nematode caused significant reductions ($P \leq 0.01$) in fresh weight of roots (23%, 30%, 27% and 49%), fresh weight of shoots (45%, 51%, 48% and 54%), plant length (25%, 31%, 34% and 27%), dry weight of shoots (40%, 51%, 47% and 53%) and dry weight of roots (24%, 48%, 26% and 41%), in the tomato cultivars Gina VF, Falat CH, Falat 111 and Karoon, respectively, in comparison to the uninoculated control. The data presented in Table 1 revealed that the treatment of the soil with the biocontrol agents or salicylic acid without nematodes significantly increased plant growth parameters.

Table 1. Effects of salicylic acid, *Trichoderma viride* and *Pseudomonas fluorescens* on growth parameters of four tomato cultivars (pooled data) infested with *Meloidogyne incognita*. C: uninoculated (negative control), N: Nematode (positive control), T: *Trichoderma viride*, SA: Salicylic acid, P: *Pseudomonas fluorescens*. Each value is the mean of four replicates in 2012 and 2013.

Tomato cultivar	Treatments	Plant length (cm)	Fresh weight (g)		dry weight (g)	
			Shoot	Root	Shoot	Root
Gina VF	C	50.5±0.57	51.3±0.31	10.44±0.10	10.27±0.32	2.23±0.31
	N	37.75*±0.50	28.1*±0.12	8*± 0.08	6.07*±0.10	1.58*±0.03
	T	52.75± 1.70	53±1.41	10.82±0.21	10.7±0.47	2.83*±0.11
	SA	54±2.16	53.25± 1.5	10.75±0.29	10.89±0.74	2.65*±0.04
	P	55.75±2.5	55±2.44	11*±0.0816	11.16±1.06	2.92*±0.04
	N+ T	40*±2.94	34.5*±0.57	7.90*±0.08	7.04*±0.65	1.42*±0.04
	N+ SA	41*±2.16	36*±0.81	7.01*± 0.11	7.32*±0.25	1.25*±0.04
	N+ P	44.25*±2.62	40.75*±1.70	8.15*±0.12	8.28±0.94	1.69*±0.03
	N+ T+ SA	50.25± 0.50	49.5±1.73	10.05*±0.03	10.02±0.76	2.01±0.01
	N+ P+ SA	50.25±0.5	50.5±1.29	10.28±0.06	10.19±1.1	2.09±0.02
	N+ P+ T	52.5±0.57	50.75±0.95	10.1±0.081	10.29±0.73	2.07±0.01
	N+ P+ T+ SA	53.5±0.57	51.5±0.57	10.35±0.07	10.45±0.52	2.2±0.03
Falat CH	C	53.5± 0.57	24.80±0.32	8.61±0.13	5.78±0.33	2.01±0.01
	N	36.75*±0.50	12*±0.81	6*± 0.08	2.8*±0.78	1.03*±0.02
	T	57.5± 2.08	28.80*±0.37	8.88±0.07	6.73±1.08	2.29±0.03
	SA	58.5± 2.88	29.41*±0.69	8.75±0.23	6.9±0.85	2.11±0.01
	P	60±2.82	33.06*±0.67	9.04*±0.03	7.76*±1.60	2.43±0.05
	N+ T	40*±1.41	16.16*±0.39	5.79*±0.17	3.86±0.17	1.01*±0.08
	N+ SA	43*± 2.94	17.34*±1.14	5.81*± 0.16	4.01±0.74	0.98*±0.01
	N+ P	46*±1.63	19.43*±2.20	6.3*±0.216	4.58±0.98	1.06*±0.02
	N+ T+ SA	53±0.81	24.55± 0.54	8*± 0.01	5.76±0.59	1.4*±0.05
	N+ P+ SA	54±1.63	23.34±1.08	8.2±0.08	5.47±0.36	1.55*±0.42
	N+ P+ T	54.5±1.73	24.45±0.39	8.01*±0.02	5.76±0.37	1.42*±0.24
	N+ P+ T+ SA	55.5±2	25.00±1.96	8.5±0.31	5.91±0.81	1.96±0.06
Falat 111	C	48.25±0.50	29.17±1.66	9.82±0.04	9.85±0.40	2.15±0.02
	N	31.75*±0.50	15*±0.81	7.15*±0.12	5.13*±0.22	1.59*±0.15
	T	51± 2.16	31.73±2.66	10.04±0.03	10.85±0.44	2.19±0.03
	SA	52±1.63	32.78±0.76	9.94± 0.12	11.23±0.71	2.17±0.02
	P	55±0.81	35.92*±2.84	10.15*±0.13	12.25*±0.94	2.23*±0.02
	N+ T	38.25*±0.95	19.53*±1.92	6.94*± 0.05	6.75*±1.08	1.56*±0.04
	N+ SA	39.25*±1.70	20.33*±1.55	6.45*±0.10	6.98*±0.14	1.46*±0.05
	N+ P	43*±2.16	23.05*±1.51	7.33*±0.17	7.94±1.17	1.65*±0.04
	N+ T+ SA	46±0.81	28.97± 0.57	9.19*±0.11	9.91±0.34	2.02*±0.03
	N+ P+ SA	48.5±1.29	28.78±1.63	9.32*±0.04	9.88±0.39	2.05±0.06
	N+ P+ T	49.25±1.70	29.6±0.29	9.18*±0.01	10.26±0.87	2.02*±0.04
	N+ P+ T+ SA	52±1.63	30.02±0.29	9.69±0.01	10.31±0.55	2.13±0.02
Karooon	C	44.5±0.57	23.97±2.53	4.27±0.17	11.2±0.46	1.05±0.11
	N	32.25*±1.70	11*±0.81	2.15*±0.12	5.21*±0.16	0.61*±0.08
	T	47.75±1.25	24.95±1.34	4.48± 0.12	11.82±0.85	1.09±0.04
	SA	48.5±0.57	25.01± 2.13	4.34±0.12	11.95±1.36	1.05±0.03
	P	51.25±0.5	27.22*±1.63	4.65*±0.129	12.87*±1.16	1.11±0.06
	N+ T	38*±0.81	15*±0.81	2.26*±0.12	7.28*±0.97	0.64*±0.26
	N+ SA	39*± 0.81	16.88*±0.73	2.15*±0.10	7.60*±0.36	0.61*±0.12
	N+ P	40*±2.16	19.29*±0.54	3.14*±0.12	9.13±0.69	0.82±0.10
	N+ T+ SA	41±2.16	21.85±1.30	3.99±0.01	10.37±0.88	0.99±0.03
	N+ P+ SA	44±0.81	22.43±0.40	4.15±0.07	10.63±0.20	1.03±0.02
	N+ P+ T	44.75±0.95	23.47±1.44	4.03±0.02	11.15±0.84	1±0.07
	N+ P+ T+ SA	45.5±0.57	24.10±1.89	4.19±0.12	11.43±0.47	1.04±0.10

* Values are significantly different from the control (Tukey test, $P \leq 0.01$).

Figure 1. Mean gall numbers of *Meloidogyne incognita*/plant on the roots of four tomato cultivars (pooled data) treated with salicylic acid, *Trichoderma viride* and *Pseudomonas fluorescens* (Treatments: T2: N, T6: N+T, T7: N+SA, T8: N+P, T9: N+T+SA, T10: N+P+SA, T11: N+P+T, T12: N+P+T+SA; N: Nematode, T: *Trichoderma viride*, SA: Salicylic acid, P: *Pseudomonas fluorescens*). Each number is a mean of four replications in two years.

Figure 2. Mean egg mass number of *Meloidogyne incognita*/plant on the roots of four tomato cultivars (pooled data) treated with salicylic acid, *Trichoderma viride* and *Pseudomonas fluorescens* (Treatments: T2: N, T6: N+T, T7: N+SA, T8: N+P, T9: N+T+SA, T10: N+P+SA, T11: N+P+T, T12: N+P+T+SA; N: Nematode, T: *Trichoderma viride*, SA: Salicylic acid, P: *Pseudomonas fluorescens*). Each number is a mean of four replications in two years.

Figure 3. Reproduction factor of *Meloidogyne incognita* on the roots of four tomato cultivars (pooled data) treated with salicylic acid, *Trichoderma viride* and *Pseudomonas fluorescens* (Treatments: T2: N, T6: N+T, T7: N+SA, T8: N+P, T9: N+T+SA, T10: N+P+SA, T11: N+P+T, T12: N+P+T+SA; N: Nematode, T: *Trichoderma viride*, SA: Salicylic acid, P: *Pseudomonas fluorescens*). Each number is a mean of four replications in two years.

The greatest increase in plant growth was obtained in cv. Falat CH treated with *P. fluorescens* CHA0 followed by SA. Fresh weights of shoots were higher in nematode-free plants in all cultivars compared to both *M. incognita*-infested plants and the above-mentioned treatments. The results showed that combined inoculation of fungi, bacteria and SA caused significant reductions ($P \leq 0.01$) in fresh weight of root (0.6%, 1%, 1% and 1.6%), dry weight of root (1.3%, 2%, 0.9% and 0.9%) in all cultivars and fresh weight of shoots (0.5 and 0.8) in two cultivars (Gina VF and Falat CH); plant length (5.9%, 3.7%, 7.7% and 2.2%), dry weight of shoots 1.75%, 2.24%, 4.6% and 2%) in all cultivars and fresh weight of shoots (0.8%, 2% and 0.1%) in three cultivars of tomato (Falat CH, Falat 111 and Karoon) increased; galling (81%, 68%, 80% and 83%), egg mass (87%, 78%, 83% and 88%) and reproduction factor (83%, 69%, 82% and 84%) were reduced compared to the inoculated control in all

cultivars (Gina VF, Falat CH, Falat 111 and Karoon, respectively) (Figure 1, Figure 2, Figure 3 and Table 1).

The data indicated that chemical inducer (salicylic acid), in combination with *T. viride* and *P. fluorescens* CHA0, stimulated plant response and increased plant growth especially in susceptible cultivars. Inoculation with the *M. incognita* race 2 caused significant reduction ($P \leq 0.01$) in plant length (25%), fresh weight of shoots (45%) and roots (23%) of tomato cv. Gina VF ($P \leq 0.01$) in comparison to the uninoculated control (Table 1). The nematode infection caused 31% reduction in plant length, 52% in fresh weight of shoot and 30% in root fresh weight of cv. Falat CH. The cv. Falat 111 exhibited significant decreases in any of the plant parameters (length (34%), fresh weights of shoots (49%) or roots (27%) at $P \leq 0.01$). In the cv. Karoon decreases in plant length (27%), fresh weight of shoots (54%) and roots (49%) were recorded. Soil treatments with *T. viri-*

de, *P. fluorescens* and foliar spray of SA gave the greatest recovery of the plant length and dry weight of shoots in all cultivars and in shoot fresh weight in three cultivars (Falat CH, Falat 111 and Karoon) compared to uninoculated control. The maximum reduction in the numbers of host root galls and egg masses was observed in Karoon cultivar representing the most susceptible cultivar.

According to growing characteristics assessments, the cultivar Gina VF, artificially inoculated with 2000 J₂ of root-knot nematode, had the highest root fresh weight, shoot fresh weight, shoot dry weight and length. Thus, this cultivar can be characterized as relatively tolerant. The lowest root fresh weight, shoot fresh and dry weight, shoot length and the maximum number of galls were counted in the cultivar Karoon that was the most susceptible cultivar. In the case of two cultivars, Falat CH and Falat 111, the Falat 111 had a greater number of galls and higher reproductive factor but its growth characters were less than Falat CH.

Discussion

Biological control of soil-borne plant pathogens and nematodes using biocontrol microorganisms is a potential non-chemical means in crop protection (Stirling, 1991).

Treatment of the soil with salicylic acid, *P. fluorescens* and *T. viride* resulted in a reduction in the galls and egg mass number of *M. incognita*. Similar results were obtained by other researchers. *Trichoderma* spp. has been reported to produce chitinases into the culture (Chet and Baker, 1981), which might help the inhibition of egg hatching. Dos Santos *et al.* (1992) reported *Trichoderma harzianum* as an effective egg parasite of *M. incognita*; the fungus was able to grow on the egg surface and penetrate the egg shell. In a study by Naserinasab *et al.* (2011), treatment of the soil with the *T. harzianum* B1 or salicylic acid resulted in a reduction in the galls, eggs and egg masses of *M. javanica*. Application of *T. harzianum*, *T. hamatum* or *T. virens* has also demonstrated potential

to suppress root-knot nematodes (Siddiqui and Shaukat, 2004).

Pseudomonas fluorescens was one of the effective agents in reducing J₂ population and prevented the formation of galls on tomato roots. Similar results have been reported (Siddiqui and Shaukat, 2003; Ali *et al.*, 2002). Siddiqui *et al.* (2006) found that there is a direct relation between the production of HCN by *P. fluorescens* CHA0 and mortality of the nematode. Akhtar *et al.* (2013) suggested that the inoculation with *P. fluorescens* increases the root and shoot length of black gram (*Vigna mungo* L.) infected with *M. incognita*. Khan and Haque (2011) reported the greatest plant growth and biomass of tobacco after treatment with *P. fluorescens* compared with *T. harzianum* and two nematicides.

Our results confirm the findings of Gharabadiyan *et al.* (2012) sustaining Gina as tolerant cultivar and Karoon as susceptible cultivar, while Falat 111 is similarly tolerant to Falat CH. The combined treatment of the soil with *T. viride*, *P. fluorescens* CHA0 and the chemical inducer salicylic acid clearly improved nematode control of the root-knot nematode *M. incognita* race 2. In addition, they stimulate plant response and increase plant growth. The study suggests that there is considerable potential for exploiting SA, *P. fluorescens*, and *T. viride* for the management of root-knot nematodes particularly in susceptible cultivars of tomato.

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Αποτελεσματικότητα σαλικυλικού οξέος, *Pseudomonas fluorescens* CHA0 και *Trichoderma viride* στην αντιμετώπιση του *Meloidogyne incognita* race 2 σε διάφορες ποικιλίες τομάτας

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Περίληψη Μελετήθηκαν οι επιδράσεις του σαλικυλικού οξέος (SA), του *Trichoderma viride* και του *Pseudomonas fluorescens* CHA0 στον κομβονηματώδη *Meloidogyne incognita* race 2 σε ανθεκτικές και ευαίσθητες ποικιλίες τομάτας (Gina VF, Falat CH, Falat 111, Karoon) κατά τα έτη 2012-2013. Χρησιμοποιήθηκαν σπορόφυτα τομάτας στο στάδιο των 4 φύλλων, τα οποία αναπτύχθηκαν σε γλάστρες με 1000 γραμμάρια αποστειρωμένου εδάφους, όπου έγινε εφαρμογή 20 ml εναιωρήματος του *T. viride* με 1×10^6 σπόρια, 30 ml του *P. fluorescens* CHA0 με 10^9 cfu/ml, 5 mM σαλικυλικού οξέος και 2000 προνυμφών δευτέρου σταδίου του νηματώδη. Έγινε εκτίμηση παραμέτρων που σχετίζονται με τον πληθυσμό των νηματωδών και την ανάπτυξη των φυτών. Οι παράγοντες βιολογικής αντιμετώπισης και το σαλικυλικό οξύ ήταν αποτελεσματικά στην καταπολέμηση των νηματωδών σε απλές ή συνδυασμένες επεμβάσεις. Μεγάλη μείωση του αριθμού των κόμβων στις ρίζες καθώς και των ωσάκκων παρατηρήθηκε μετά από τη συνδυασμένη επέμβαση του σαλικυλικού οξέος με τους βιολογικούς παράγοντες. Η μεγαλύτερη αύξηση στην ανάπτυξη των φυτών επιτεύχθηκε στην ποικιλία Falat CH μετά από επέμβαση με σαλικυλικό οξύ, ακολουθούμενη από τις επεμβάσεις με το *P. fluorescens* CHA0 και το *T. viride*. Ο μεγαλύτερος αριθμός κόμβων καταγράφηκε στην ποικιλία Karoon και ακολούθησαν οι ποικιλίες Falat 111, Gina VF και Falat CH. Η επέμβαση με *P. fluorescens* CHA0 προκάλεσε τη μεγαλύτερη αύξηση σε νωπό και ξηρό βάρος ρίζας, νωπό και ξηρό βάρος βλαστών και στο μήκος των φυτών σε όλες τις επεμβάσεις απουσία νηματωδών. Τα αποτελέσματα έδειξαν ότι το σαλικυλικό οξύ, σε συνδυασμό με τους βιολογικούς παράγοντες (*T. viride* και *P. fluorescens* CHA0), διήγειραν και τελικά αύξησαν την ανάπτυξη του φυτού.

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