بررسی تعامل بین نماتد ریشه‌گرهی *Meloidogyne javanica* و *Fusarium oxysporum* f. sp. *radicis-cucumerinum* روی برخی ارقام خیار تحت شرایط گلخانه*

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چکیده

تعامل قارچ عامل بیماری پوسیدگی ریشه و ساقه خیار *Fusarium oxysporum* f. sp. *radicis-cucumerinum* و نماتد *Meloidogyne javanica* روی سه رقم خیار خصیب، نگین و دستجردی در آزمایشی به صورت طرح کاملا تصادفی در قالب فاکتوریل با ۱۴ تیمار در چهار تکرار شامل شاهد، مایه‌زنی قارچ به تنهایی، نماتد به تنهایی در چهار سطح ۱۵۰۰، ۳۰۰۰، ۴۵۰۰ و ۶۰۰۰ لارو نماتد در هزار گرم خاک و مایه زنی قارچ به دو صورت همزمان و یک هفته بعد از مایه‌زنی با نماتد در شرایط گلخانه بررسی گردید. در مایه‌زنی همزمان قارچ و نماتد با سطح جمعیتی ۶۰۰۰ لارو، شاخص گال ریشه نسبت به تیمار های تلقیح شده با نماتد به تنهایی ۲۳/۰۷ درصد کاهش یافت. نتایج نشان داد که بیشترین میانگین تعداد تخم، تعداد کیسه تخم در گرم ریشه و شاخص گال به ترتیب ۳۱۵۵/۲، ۱۵/۳۰ و۴/۳۳ مربوط به مایه‌زنی گیاه با ۶۰۰۰ لارو به تنهایی بود. حضور نماتد (۶۰۰۰ لارو) باعث افزایش ۶۷/۸۱ درصدی شاخص پژمردگی نسبت به مایه‌زنی با قارچ به تنهایی شد. همچنین به طور میانگین در ارقام خیار در تیمار مایه‌زنی قارچ بعد از نماتد شاخص پژمردگی نسبت به مایه‌زنی همزمان ۳۵/۰۳ درصد در سطح جمعیتی شش لارو در گرم خاک افزایش نشان داد، همچنین این تیمار موجب کاهش ۲۷/۲۳ درصدی وزن تر شاخساره گیاه و افزایش ۳/۵ برابری شاخص پژمردگی ارقام خیار نسبت به شاهد شد. نتایج نشان داد که حضور نماتد و قارچ یک عامل تشدید کننده بیماری محسوب می‌شود که این مورد در رقم نگین کاملا مشخص بود.

کلیدواژه: خیار، قارچ، تعامل، متغیر نماتد، پارامترهای رشد گیاه

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Investigation on the interaction between the root-knot nematode *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *radicis-cucumerinum* on some cucumber cultivars under greenhouse condition

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Abstract

In order to study the interaction of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* and *Meloidogyne javanica* a survey on three cucumber cultivars was conducted in the greenhouse condition. The experiments were laid out in a factorial experiment based on completely randomized design (CRD) within 14 treatments including control, fungi alone, nematode alone in four inoculations level viz. 1500, 3000, 4500 and 6000 J2s, fungus + nematode simultaneously, fungus a week after nematode inoculation with 4 replications. Simultaneous fungus and nematode (6000 J2s) inoculation resulted in a %23.07 reduction in nematode gall index. The results showed that the highest mean egg number and number of egg sac per gram of root and gall index in the three cucumber cultivars were 3155.2, 15.30 and 4.33, respectively, for inoculation of the plant with 6000 nematode juvenile. The presence of nematodes (6000 J2s) increased the wilting index by 67.81% compared to fungus inoculation alone, and also, in fungal inoculation treatments, after the nematode inoculation (6000 J2s), the wilting index increased by 35.03% compared to the simultaneous inoculation. The results indicated that decreasing of 27.23% fresh shoot mass and the increasing of 3.5-fold wilting index of the cucumber cultivars to control caused by *M. javanica* at 6000 J2 → *F. oxysporum* f. sp. *radicis-cucumerinum* (one week later of inoculation) after 45 days. In conclusion, the results indicated that simultaneous infection of *M. javanica* and *F. oxysporum* f. sp. *radicis-cucumerinum* have synergistic effect on disease which was quite clear in Negin cultivar.

Keywords: cucumber, fungus, interaction, nematode variable, plant growth parameters

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Introduction

Cucumber (*Cucumis sativus* L.) is the main greenhouse crop, especially in Yazd Province of Iran. Root and stem rot fungus (*Fusarium oxysporum* f. sp. *radicis-cucumerinum*) and the root-knot nematodes *Meloidogyne javanica* (Treub 1885) Chitwood, 1949 are the most destructive pathogens that threatening glasshouse cucumber cultivation in Iran (Moosavi *et al.* 2006, Shahriari *et al.* 2011). The occurrence and severity of the resulting diseases have increased substantially over the last five years and are causing yield-limiting factors in greenhouse cucumber production (Mohamadian-Sarcheshmeh & Ahmadi 2014). Crops infected with root-knot nematodes are usually subjected to various nematodes-induced modifications. These can vary from localized forms of tissue damage to overall systemic effects such as retarded plant growth. Furthermore, these changes can influence infection rates of other soil-borne pathogens (Back *et al.* 2002). There are a number of reports, which clearly illustrate that nematode damage plays a role in the establishment and development of diseases caused by soil-borne pathogens (Gheysen & Mitchum 2011, Saeedizadeh *et al.* 2008, Sahebani *et al.* 2008, Shokoohi *et al.* 2003). Interactions between root-knot nematode and *Fusarium* wilt have been studied and documented in different crops, including alfalfa (Griffin & Thyr 1988), bananas (Jonathan & Rajendran 1998), beans (Faraji *et al.* 2007, France & Abawi 1994), carnations (Schindler *et al.* 1961), chickpea (Khan & Hosseini-Nejad 1991, Kumar *et al.* 1988), coffee (Bertrand *et al.* 2000), cotton (Davis *et al.* 1996, Imani *et al.* 2014), green bean (Imani *et al.* 2014), lentil (De *et al.* 2001), melon (Shokoohi *et al.* 2003), olive (Saeedizadeh *et al.* 2008), pea (Maheswari *et al.* 1997), tobacco (Porter & Powell 1967) and tomato (Abawi & Barker 1984, Sahebani *et al.* 2008). In all these interactions, root-knot nematode and *Fusarium* spp. increased fungus pathogenicity. Furthermore, nematode density and time of fungus inoculation influenced plant growth and nematode indices. In this study, the interaction between root-knot nematode and *F. oxysporum* f. sp. *radicis-cucumerinum* was investigated on some cultivars of cucumber in Yazd Province, Iran. The aims of this study include achieving greater recognition of cucumber pathogens, acquiring information on these interactions, investigating the effects of single vs. Simultaneous infection by the studied pathogens and providing insight into the control of these pathogens.

Materials and methods

Nematode inoculum preparation

Meloidogyne javanica was isolated from infected roots collected from different cucumber cultivars *viz.* Khasib, Negin, and Dastjerdi in cities of Yazd, Taft, Meybod, Sadoogh (Yazd Province), Iran. The nematode isolates and perineal patterns were prepared (Taylor & Netscher 1974) and identified as *M. javanica* according to morphological and morphometric characteristics (Eisenback 1985). The isolated population of Negin cultivar was used in the experiment. The single egg mass method was used to rear nematode population on Rutgers tomato cultivar after which root-knot nematode was further mass produced in a greenhouse at 23-28°C (Moosavi *et al.* 2006). Nematode eggs were extracted from galled roots (Hussey & Barker 1973). The roots were washed with tap water and cut into two to three centimeters pieces, then the roots were shaken in a bottle containing 0.5% sodium hypochlorite (NaClO) for five minutes. Extracted eggs were rinsed with tap water in order to remove the NaClO residue (Hussey & Barker 1973). Furthermore, the number of J2s was counted and the mean value (of three replicates) calculated per 10 ml sterile water. Depending on the treatment, inoculum was 1500, 3000, 4500 and 6000 J2s in 10 ml sterile water for each pot .

Fungal isolates

Samples of infected roots of greenhouse cucumbers were cultured on potato dextrose agar (PDA) medium and incubated at 25°C for five days. Fifteen isolates of fungus were obtained from infected roots of mature cucumbers that showed symptoms of the root and stem rot and/or *Fusarium* wilt symptoms, in different greenhouses in Yazd Province, Iran. Eight of these isolates were identified by pathogenicity tests and valid key as *F. oxysporum* f. sp. *radicis- cucumerinum* (Lesli *et al.* 2006, Nelson *et al.* 1993, Vakalounakis *et al.* 2005. Then they were maintained on potato dextrose agar (PDA) at 6°C. . The inoculum was then grown on potato dextrose broth (PDB) (Vakalou-

nakis 2005) using 200 ml Erlenmeyer flask in a rotatory shaker for seven days at 20°C with no direct exposure to sunlight. The pathogenicity of each isolate was tested on seedlings of different cucumber cultivars at the third leaf stage using the root dipping technique (Vakalounakis *et al.* 2005). The pathogenicity of each isolate was tested on seedlings of the four differential cucurbit species: *C. melo* (melon, cv. Moulkeiko), *C. sativus* (cucumber, cv. Knossos), *L. aegyptiaca* (sponge gourd) and *Cucurbita maxima* × *C. moschata* (pumpkin) at the two-true-leaf stage using the root dipping technique (Vakalounakis, 1996). Final observation on disease development was made about 30 days after inoculation. To confirm infection of cucurbit plants by *F. oxysporum* , isolations were made from plants with or without symptoms. Pathogenicity tests were conducted in a complete randomized design. For each isolate and differential host combination, eight replicate plants were used. Tests were carried out at least twice. Disease severity was assessed with a 0–3 rating visual scale, as follows: 0, no symptoms; 1, light or moderate rot on taproot, secondary roots and crown, light vascular discoloration in the stem; 2, severe rot on taproot, secondary roots and crown, with or without wilting and stunting, vascular discoloration in the stem; and 3, dead seedlings. Within each *forma specialis* and differential host, disease indices were compared using the least significant difference test ($P \leq 0.05$)

Greenhouse experiments

Three prevailing cultivars of cucumber (Khasib, Negin and Dastjerdi) were subjected to experimental studies. The cucumber seeds were surface sterilised with 1.2% NaOCl for five minutes after which six seeds were sown in an eight inches deep pot containing 1000 cm^3 sterilized soil. The soil consisted of a mixture of greenhouse soil (30% silty clay and 70% organic matter) and sand (1:1 v/v) (Shokoohi *et al.* 2003). Plants at the third leaf stage were removed from the soil and the roots rinsed with Distilled water and then dipped for 20 min in a microconidial suspension of fungus containing approximately 10^6 conidia/ml, cultured in potato dextrose broth (PDB) for seven days at 28°C, under continuous light. Seedlings were inoculated with an inoculum of both pathogens including second stage juveniles of the nematode (J2s), or microconidial suspension of fungus as well as distilled water as control (Oka *et al.* 1999). Each plant was inoculated at the third leaf stage with nematode using the technique developed by Hussey and Barker method (Hussey & Barker 1973). For the experiment, each pot was infested with five nematode inoculum levels (zero, 1500, 3000, 4500 and 6000 J2s) by distributing inoculum within two dibble holes (3-cm-deep) at the base of each plant and then covering with soil. For inoculation of fungi and nematodes simultaneously, initially, inoculation with nematode was carried out and then with the fungus according to the methods are described. Experiments were carried out in a greenhouse with air temperature ranging from 23°C to 28°C. The experimental setup was based on a factorial completely randomized designed with a total of 14 treatments and 4 replications. This experiment comprises 14 treatments including control, fungi alone, nematode alone in four inoculations level viz. 1500, 3000, 4500 and 6000 J2s, fungus + nematode simultaneously, fungus a week after nematode inoculation with 4 replications. Both plant growth and nematode indices were evaluated after harvesting the plants. Plant growth indices included fresh and dry shoot weight and root weight and length 45 days after fungus inoculation. Furthermore, the shoot length was measured and the wilting index was scored after 10, 20, 30 and 45 days of inoculation on 1-6 scale (Marley & hilock 1996) where: 1 = no visible symptom; 2 = epinasty and chlorosis/wilting of primary leaves; 3 = chlorosis/wilting of 2nd and 3rd leaves may be lost, primary leaves may be lost; 4 = chlorosis above 3rd leaves, 2nd and 3rd leaves may be lost; chlorosis/wilting of whole plant and 6 = plant completely desiccated. Nematode related indices included the gall index, number of egg mass, number of egg in one gram of root, as well as the reproduction factor (Rf) (Walters *et al.* 1999) (Hussey & Barker 1973). The gall index was on a scale of 0 to 5 (Taylor & Sasser 1978), where 0= roots without egg mass and/or gall; 1= roots with 1-2 egg masses and/or galls; 2= roots with 3-10 egg masses and/or galls; 3= roots with 11-30 egg masses and/or galls; 4= roots with 31-100 egg masses and/or galls; and 5= roots with more than 100 egg masses and/or galls.

Statistical analysis

Data generated from the measured variables were subjected to analysis of variance (ANOVA)

and means were compared with Duncan's multiple range tests using IBM® SPSS® Statistics software version 22 (IBM SPSS Statistics for Windows, Version 22.0., Armonk, NY, USA). The experiment was conducted in the greenhouse and repeated once. The independent samples t-Test compared the means of two independent groups (two experiments) in order to determine whether there was statistical evidence that the associated population means were significantly different. The results of the independent-sample t-Test showed that two experiments have not shown significant differences at $p \leq 0.05$. T-test for equality of means and Levene's test for equality of variances was done.

Results

Plant Growth parameters

The results of independent-sample T-test showed that two groups (two trials) have not shown significant differences ($p \leq 0.05$). The interaction between the cucumber cultivars (Khasib, Negin and Dastjerdi), *M. javanica* and *F. oxysporum* f. sp. *radicis-cucumerinum* was evaluated either individually or in combination. Results showed that all of the treatments resulted in significant differences ($P \leq 0.05$) compared to the control (untreated) plants. The shoot length at 10, 20, 30 and 45 days after fungus inoculation (Table 1) showed a significant difference compared with the untreated plants ($p \leq 0.05$). With increasing the nematode initial population, growth indices of plants decreased compared with the control. The lowest index of plant growth was related to the presence of the fungus and 6000 juveniles treatment. Fungal inoculation alone and without inoculation with nematode led to decreased plant growth indices compared with the control. In presence of the nematode, a significant reduction of shoot length, fresh and dry weight of cucumber cultivars was caused by application of the fungus after nematode treatment. The interaction between the nematode and fungus was more evident when applied in combination. However, the Negin cultivar showed significant reduction in the root and shoot length ($P \leq 0.05$) compared with the control. This plant height reduction expressed by a delayed growth reflected the presence of a synergistic interaction within this complex. The shoot dry and fresh weight of cucumber cultivars (Table 1) also showed a significant interaction ($P \leq 0.05$) between the nematode and fungus. This interaction was most evident in the Negin cultivar where a significant reduction of shoot fresh weight, of about 32.2% was recorded compared to the non-inoculated plants of about 9.45% without nematode infestation. However, for the rest of initial population of the root-knot nematode and fungal inoculations, these parameters showed significant reduction in growth parameters (Table 1).

Nematode growth indices

A significant interaction ($P \leq 0.05$) was observed when both pathogens were present. All cucumber cultivars infested with *M. javanica* showed gall development on the roots. The loweest gall index as observed on plants inoculated by a mixture of *F. oxysporum* f. sp. *radicis-cucumerinum* and *M. javanica* indicated the presence of an interaction within this parasitic complex. It was also revealed that cucumber cultivars inoculated only with root-knot nematode had a significantly higher galling index than those of the non-inoculated control plants and inoculated plants with fungus. The highest gall index was 5 for Negin and Khasib in the application of 6000 J2s and the lowest was for Dastjerdi in the application of 1500 J2s of *M. javanica* only. However, compared to the others cultivars, the Khasib cultivar showed the highest gall index (3.75) and Dastjerdi cultivar of cucumber showed the lowest gall index (2.25) in a complex interaction (Table 3). The gall index ranged between 2- 4.00 when both pathogens were present. The egg mass number in the presence of *F. oxysporum* f. sp. *radicis-cucumerinum* showed a significant reduction ($P \leq 0.05$). Egg masses were observed on all cucumber cultivars infested with *M. javanica* and their number varied depending on fungal treatments (Table 3). A non-synergistic interaction was observed in the studied parasitic complex, via this parameter, as also recorded for the galling index. Reproduction factor (Rf) ranged between 1.28 - 6.58 in the presence of root-knot nematode only and was the highest when 3000 juvenile of *M. javanica* was inoculated in Khasib cultivar (Table 4). The presence of *F. oxysporum* f. sp. *radicis- cucumerinum*, especially simultaneous inoculation with *M. javanica* on Dastjerdi cultivar (6000 J2s) caused the lowest of nematode reproduction factor (Table 3).

Table 1. Interaction between *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *radicis-cucumerinum* on shoot fresh and dry weights (g) , shoot length (cm), root fresh and dry weights (g) of some cucumber cultivars.

Treatments	Shoot fresh weight (g)			Shoot dry weight (g)			shoot length (cm)			Fresh weight of root (g)			Dry weight of root (g)		
	K[1]	N	D	K	N	D	K	N	D	K	N	D	K	N	D
Control	12.31[a2]	11.85[a]	11.61[a]	4.13[a]	4.04[a]	4.01[a]	73.20[a]	67.40[a]	86.05[a]	5.07[cd]	4.89[b]	5.26[cd]	0.73[d]	0.70[bc]	0.77[de]
Fungus inoculation	11.71[c]	10.73[c]	9.00[f]	4.02[de]	3.85[bc]	3.88[e]	70.67[c]	58.30[d]	83.05[bc]	3.95[f]	3.71[fg]	5.23[de]	0.50[fg]	0.44[hi]	0.77[de]
Nematode (1500 juvenile) alone	12.08[b]	11.73[b]	10.90[c]	4.09[b]	4.03[a]	3.88[e]	69.82[c]	64.00[b]	82.82[c]	5.02[d]	4.75[c]	5.49[bc]	0.72[d]	0.67[d]	0.82[bc]
Nematode (1500 juvenile) →Fungus	11.51[c]	10.64[e]	9.22[e]	4.00[e]	3.83[c]	3.78[d]	68.10[d]	51.25[g]	84.32[b]	3.94[fg]	3.71[fg]	5.23[de]	0.48[gh]	0.44[hi]	0.77[de]
Nematode (1500 juvenile) + Fungus	11.74[c]	10.81[cd]	8.60[h]	4.04[cd]	3.86[b]	3.81[d]	71.02[b]c	55.20[f]	86.35[a]	3.99[ef]	3.90[de]	5.05[e]	0.50[f]	0.48[ef]	0.73[e]
Nematode (3000 juvenile) alone	10.91[d]	9.94[f]	10.89[c]	3.89[f]	3.71[d]	3.54[f]	65.15[e]	56.70[e]	70.47[d]	5.16[bc]	4.90[b]	5.54[b]	0.74[c]	0.69[e]	0.83[b]
Nematode (3000 juvenile) →Fungus	10.42[f]	8.83[i]	8.43[i]	3.79[h]	3.50[f]	3.44[h]	61.87[f]	49.35[h]	68.00[e]	3.89[fg]	3.60[gh]	5.18[e]	0.48[gh]	0.41[jk]	0.75[de]
Nematode (3000 juvenile) + Fungus	10.68[e]	9.08[h]	8.47[i]	3.84[g]	3.55[e]	3.45[gh]	64.25[e]	50.95[g]	70.025[d]	3.80[hi]	3.80[ef]	5.22[de]	0.46[i]	0.47[fg]	0.76[de]
Nematode (4500 juvenile) alone	10.17[g]	9.05[h]	10.35[d]	3.75[i]	3.55[e]	3.57[e]	60.10[g]	48.62[h]	67.45[e]	5.25[b]	5.01[ab]	5.68[ab]	0.77[b]	0.72[ab]	0.86[ab]
Nematode (4500 juvenile) →Fungus	9.65[hi]	7.96[k]	10.41[d]	3.67[k]	3.35[i]	3.48[g]	57.50[hi]	40.27[k]	65.92[f]	3.84[gh]	3.59[gh]	4.83[f]	0.47[hi]	0.42[ijk]	0.68[f]
Nematode (4500 juvenile) + Fungus	9.80[h]	8.15[j]	8.71[g]	3.68[ik]	3.38[gh]	3.49[g]	59.62[g]	42.60[j]	68.05[e]	3.74[ij]	3.83[ef]	5.29[cd]	0.55[e]	0.47[fg]	0.78[d]
Nematode (6000 juvenile) alone	10.15[g]	9.23[g]	11.14[b]	3.71[j]	3.57[e]	3.55[ef]	57.92[h]	46.90[i]	61.15[g]	5.35[a]	5.12[a]	5.81[a]	0.99[a]	0.74[a]	0.88[a]
Nematode (6000 juvenile) →Fungus	9.50[j]	8.03[k]	8.50[hi]	3.49[m]	3.36[hi]	3.44[h]	55.42[j]	37.60[l]	61.25[h]	3.62[k]	3.48[h]	4.72[f]	0.42[k]	0.40[k]	0.67[f]
Nematode (6000 juvenile) + Fungus	9.66[hj]	8.20[j]	8.58[h]	3.64[l]	3.40[g]	3.48[g]	56.42[ij]	39.97[k]	64.15[g]	3.67[jk]	3.77[f]	4.75[f]	0.45[j]	0.45[gh]	0.67[f]

1-K = Khasib, N = Negin, D = Dastdgerdi, Nematode+Fungus = Simultaneous inoculation, Nematode→Fungus = Fungus inoculation one week after nematode.
2-Significant differences are denoted by different letters within each column (Duncan's multiple range test) (P ≤0.05).

Table 2. Interaction between *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *radicis-cucumerinum* on wilting index of some cucumber cultivars.

Treatments	Wilting index 10 days after fungus inoculation			Wilting index 20 days after fungus inoculation			Wilting index 30 days after fungus inoculation			Wilting index 45 days after fungus inoculation		
	K[1]	N	D	K	N	D	K	N	D	K	N	D
Control	1.00[a2]	1.00[d]	1.00[b]	1.00[c]	1.00[e]	1.00[e]	1.00[d]	1.00[g]	1.00[a]	1.00[e]	1.00[g]	1.00[e]
Fungus inoculation	1.00[a]	2.00[b]	1.00[b]	1.00[c]	3.00[c]	1.00[e]	2.00[c]	3.00[e]	2.00[b]	2.00[d]	3.00[e]	2.00[d]
Nematode (1500 juvenile) alone	1.00[a]	1.00[d]	1.00[b]	1.00[c]	1.00[e]	1.00[e]	1.00[d]	1.00[g]	1.00[c]	1.00[e]	1.00[g]	1.00[e]
Nematode (1500 juvenile) →Fungus	1.00[a]	2.00[b]	1.00[b]	1.00[c]	3.00[c]	1.00[e]	2.00[c]	4.00[c]	2.00[b]	2.00[d]	4.00[c]	2.00[d]
Nematode (1500 juvenile) + Fungus	1.00[a]	1.00[d]	1.00[b]	1.00[c]	2.00[d]	1.00[e]	2.00[c]	3.00[e]	2.00[a]	2.00[d]	3.00[e]	2.00[d]
Nematode (3000 juvenile) alone	1.00[a]	1.00[d]	1.00[b]	2.00[b]	2.00[d]	2.00[b]	2.00[c]	2.00[f]	2.00[b]	2.00[d]	2.00[f]	2.00[d]
Nematode (3000 juvenile) →Fungus	1.00[a]	2.00[b]	1.00[b]	1.00[c]	3.00[c]	1.00[e]	2.00[c]	4.00[c]	2.00[b]	3.00[c]	4.00[c]	3.00[c]
Nematode (3000 juvenile) + Fungus	1.00[a]	1.00[d]	1.00[b]	2.00[b]	2.00[d]	2.00[b]	3.00[b]	3.00[e]	2.00[b]	3.00[c]	3.00[e]	3.0[c]
Nematode (4500 juvenile) alone	2.00[a]	1.00	1.00[b]	2.00[b]	2.00[d]	2.00[b]	3.00[b]	3.00[e]	2.00[b]	3.00[c]	3.00[e]	3.00[c]
Nematode (4500 juvenile) →Fungus	1.00[a]	2.00[b]	1.00[b]	1.00[c]	4.00[b]	1.00[e]	2.00[c]	5.00[b]	2.00[b]	3.00[c]	5.0[b]	3.00[c]
Nematode (4500 juvenile) + Fungus	1.00[a]	2.00[b]	1.00[b]	2.00[b]	3.00[c]	2.00[b]	3.00[b]	4.00[c]	2.00[b]	3.00[c]	4.00[c]	3.00[c]
Nematode (6000 juvenile) alone	2.00[a]	2.00[b]	1.00[b]	2.00[b]	3.00[c]	2.00[b]	3.00[b]	3.00[c]	3.00[a]	3.00[c]	3.00[e]	3.00[c]
Nematode (6000 juvenile) →Fungus	2.00[a]	3.00[a]	1.00[b]	3.00[a]	5.00[a]	3.00[a]	3.50[a]	6.00[a]	3.00[a]	4.00[a]	6.00[a]	3.50[b]
Nematode (6000 juvenile) + Fungus	1.00[a]	1.50[c]	1.50[a]	2.00[b]	3.00[c]	2.00[b]	3.00[b]	3.50[d]	3.00[a]	3.50[b]	3.50[d]	3.00[c]

1- k = Khasib, N = Negin, D = Dastdgerdi, Nematode−Fungus = Simultaneous inoculation, Nematode→Fungus = Fungus inoculation one week after nematode.
2- Significant differences are denoted by different letters within each column (Duncan's multiple range test) (P ≤0.05).

Table 3. Interaction between *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *radicis-cucumerinum* on gall and RF indices, egg sac and egg indices of some cucumber cultivars.

Treatments	Gall index			Rf			Number of egg sac per gram of root			Number of egg per gram of root		
	K[1]	N	D	K	N	D	K	N	D	K	N	D
Control	0.00[d2]	0.00[e]	0.00[c]	0.00[l]	0.00[k]	0.00[k]	0.00[k2]	0.00[j]	0.00[j]	0.00[j]	0.00[l]	0.00[l]
Fungus inoculation	0.00[d]	0.00[e]	0.00[c]	0.00[l]	0.00[k]	0.00[k]	0.00[k]	0.00[j]	0.00[j]	0.00[j]	0.00[l]	0.00[l]
Nematode (1500 juvenile) alone	3.00[c]	3.00[c]	2.00[b]	5.11[b]	3.01[d]	2.40[a]	4.25[b]	3.50[i]	1.50[g]	1527.20[i]	900.00[i]	718.50[e]
Nematode (1500 juvenile) →Fungus	3.00[c]	3.00[c]	2.00[b]	3.90[d]	2.06[g]	0.68[e]	3.75[i]	3.00[k]	1.00[h]	1486.40[ij]	788.00[j]	260.00[j]
Nematode (1500 juvenile) + Fungus	3.00[c]	2.00[d]	2.00[b]	3.50[f]	1.66[j]	0.60[g]	3.50[j]	2.75[j]	1.00[h]	1317.60[k]	626.40[k]	228.00[k]
Nematode (3000 juvenile) alone	4.00[b]	4.00[b]	3.00[a]	6.58[a]	4.09[b]	2.02[b]	8.25[e]	7.25[f]	2.25[e]	3825.60[d]	2383.20[e]	1178.40[b]
Nematode (3000 juvenile) →Fungus	3.00[c]	3.00[c]	2.00[b]	3.76[de]	2.83[e]	1.03[c]	7.75[f]	6.75[g]	1.75[e]	2901.60[g]	2184.00[f]	799.20[d]
Nematode (3000 juvenile) + Fungus	3.00[c]	3.00[c]	2.00[b]	3.22[g]	2.76[e]	0.89[d]	7.50[g]	5.75[h]	1.50[f]	2544.00[h]	2181.60[f]	708.00[f]
Nematode (4500 juvenile) alone	5.00[a]	4.00[b]	3.00[a]	4.75[c]	3.59[c]	1.80[b]	19.25[a]	10.75[e]	3.25[b]	4075.20[c]	3081.60[d]	1551.20[a]
Nematode (4500 juvenile) →Fungus	4.00[b]	3.00[c]	2.00[b]	3.08[h]	2.22[i]	0.80[h]	12.00[c]	8.21[d]	1.77[d]	3614.40[e]	2606.40[g]	943.20[g]
Nematode (4500 juvenile) + Fungus	4.00[b]	3.00[c]	2.00[b]	2.66[i]	1.96[i]	0.61[i]	11.50[d]	7.80[e]	1.75[e]	3204.00[f]	2368.80[h]	694.40[g]
Nematode (6000 juvenile) alone	5.00[a]	5.00[a]	3.00[a]	4.14[d]	3.00[a]	1.28[ef]	20.50[a]	19.73[a]	5.16[a]	4651.20[a]	3374.40[a]	1440.00[c]
Nematode (6000 juvenile) →Fungus	4.00[b]	4.00[b]	2.00[b]	2.50[j]	1.92[h]	0.69[ij]	14.25[b]	13.00[b]	1.78[d]	3147.20[b]	3187.20[c]	1147.20[b]
Nematode (6000 juvenile) + Fungus	4.00[b]	4.00[b]	2.00[b]	2.06[k]	1.44[f]	0.57[j]	12.50[c]	10.50[c]	1.75[e]	3384.00[e]	2356.80[b]	1008.00[i]

1- K = Khasib, N = Negin, D = Dastgerdi, Nematode+Fungus = Simultaneous inoculation, Nematode→Fungus = Fungus inoculation one week after nematode.
2- Significant differences are denoted by different letters within each column (Duncan's multiple range test) ($P \leq 0.05$).

Wilt index (WI)

The wilting index was scored after 10, 20, 30 and 45 days of inoculation on 1-6 scale (Marley & hilock 1996. A synergism type of interaction was observed in the studied parasitic complex, via this parameter. The highest wilting index as observed on plants inoculated with a mixture of *F. oxysporum* f. sp. *radicis-cucumerinum* and *M. javanica* indicated the presence of a synergistic interaction within this parasitic complex. The highest wilting index was 5 for Negin in the applications of 6000 J2s and 4500 J2s (Table 2). In nematode inoculation treatments alone, with the increase of the nematode population level from 1500 to 4500, the wilting index increased by 200%. While with the increase in population from 4500 larvae to 6000 larvae, the wilting index remained constant. The wilting index ranged between 2.00 to 5.00 when both pathogens were present.

Discussion

The interaction between *M. javanica* and *F. oxysporum*. f. sp. *radicis-cucumerinum* on cucumber was studied for the first time in the present study. The results indicated the effect of individual and/or combined inoculations of both pathogens on plant growth parameters and nematode growth and reproduction indices. Plant height was shown to be negatively affected by the presence of *M. javanica* and *F. oxysporum*. f. sp. *radicis-cucumerinum* as a synergistic effect seemed to occur. In some other fungi and nematode interactions, significant growth reductions, if compared to the control, were recorded (Anjos *et al.* 2010, Atkinson 1892, Khan & Hosseini-Nejad 1991, Shokoohi *et al.* 2003). Even only *M. javanica* infestation caused significant reductions in shoot growth parameters when compared with the control treatments. This decrease was probably due to plant physiological disorders. However, when *F. oxysporum* was inoculated following nematode inoculation, the decline in the growth of the aerial part was greater. In fact, *F. oxysporum*. f. sp. *radicis-cucumerinum* isolate was more aggressive when inoculated following the nematode inoculation. The highest wilting index as observed on plants inoculated with a mixture of *F. oxysporum* f. sp. *radicis-cucumerinum* and *M. javanica* indicated the presence of a synergistic interaction within this parasit-

ic complex. In fungal inoculation treatments, after the nematode inoculation, the wilting index increased. The reason for this was more the fungus entry and increased wilting caused by it. The highest wilting index was 5 for Negin in the applications of 6000 J2s and 4500 J2s. The reason for the greater rate of wilting was its greater susceptibility to fungus. In nematode inoculation treatments alone, with the increase of the nematode population from 1500 larvae to 4500 larvae, the wilting index increased by 200%. While with the increase in population from 4500 larvae to 6000 larvae, the wilting index remained constant. This confirmed that the nematode population could increase the wilting index to some extent. Also, nematode as a pathogen alone could cause wilting on plants. The nematode predisposed the fungal infection as also observed on banana plants in the presence of *M. incognita* and *F. oxysporum* f. sp. *cubense* where the severity of the banana wilt disease was enhanced in mixed infections (Jonathan & Rajendran 1998). Synergistic interactions were indicated by an additive negative effect on dry and fresh weight of the aerial part with mixed inoculations was recorded in *M. javanica* and *F. oxysporum* f. sp. *radicis-cucumerinum* interaction. Similar results were obtained by Khan and Hosseini-Nejad (1991) on chickpea where *M. javanica* caused significant reductions in dry weight if compared to uninoculated control plants; however, with the addition of *F. oxysporum* f. sp. *ciceri*, the decline was greater (Khan & Hosseini-Nejad 1991). The similar results obtained by some scientists (Fazal *et al.* 1994, Khan & Akram 2000, Reddy *et al.* 1979). The present study also showed that the wilt severity was strongly affected by fungal inoculations followed by the application of root-knot nematodes. In fact, *F. oxysporum*. f. sp. *radicis-cucumerinum* isolate was more aggressive when inoculated following the nematode inoculation. The juvenile stage could make host tissues easier to penetrate by fungal pathogens. Furthermore, vascular pathogens alter the normal translocation of water in the plant by clogging the vessels with fungal structures by the accumulation of metabolic products from the pathogen, increased activity of toxins produced by the pathogen and/or as a result of the production of tyloses of the plant (Khan & Hosseini-Nejad 1991). These symptoms appear and increase disease severity in combined inoculations with fungal pathogens and nematodes generally occurred in synergistic interactions. France and abavi (1994) studied four genotypes of bean and indicated that severe infection of the roots by *M. Incognito* increased the severity of Fusarium wilt disease (France & above 1994). In addition, wilting severity reported in cotton by the application of *M. incognita* and *F. oxysporum* f. sp. *vasinfectum* (Martin *et al.* 1994). Interaction between *M. javanica* and *F. oxysporum* f. sp. *ciceri* increased chlorosis of leaves about 10% to 100% in chickpea (Maheswari *et al.* 1997). The present study showed that *M. javanica* development and reproduction depended on the tested fungal treatments. Root galling was also significantly influenced by the interaction of two pathogens on three varieties. In fact, the important gall index was associated with an increased egg mass formation and female fecundity and this generally occurred in interactions in the bipartite complex. The study showed that the synergistic interaction occurred between the two pathogens on cultivars. The synergistic interaction was evident in all parameters except nematode indices. Physiological changes induced on roots infected with the nematode may be the cause of plant sensitivity to the fungus in the presence of nematode. Fungal penetration and colonization of the root system enhanced by the establishment of the nematode may account for reductions in the growth and development of the hosts as well as for differences in the gall index recorded on nematode-infected plants. The present results also showed that for a certain complex, the lowest galling index was associated with reduced egg mass production. Similarly, Daami-Remadi *et al.* (2009) found that potato infestation by *Meloidogyne* was greatest when the nematode occurred alone, whereas in the presence of *F. oxysporum* reproduction and root galling was significantly reduced (Daami-Remadi *et al.*, 2009). In conclusion, the present study revealed that the contribution of *M. javanica* in the *Fusarium* wilt disease and the consequent reduction of cucumber growth depending on the type of inoculation of the fungus and used nematode density. The nematode development and reproduction were also shown to be strongly affected by the fungus. For better understanding the relationship between *M. javanica* and isolates of *F. oxysporum*. f. sp. *radicis-cucumerinum*, filed study should be done in the future studies as well as histological studies.

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