RESEARCH

THE EFFECTS OF DIFFERENT FUNGICIDES ON THE VIABILITY OF ENTOMOPATHOGENIC NEMATODES *Steinernema feltiae* (FILIPJEV), *S. carpocapsae* WEISER, AND *Heterorhabditis downesi* STOCK, GRIFFIN & BURNELL (NEMATODA: RHABDITIDA) UNDER LABORATORY CONDITIONS

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To increase our knowledge on the susceptibility of entomopathogenic nematodes (EPN) species to agrochemicals, the compatibility of the infective juveniles (IJ) of the entomopathogenic nematodes *Steinernema feltiae*, *S. carpocapsae*, and *Heterorhabditis downesi* with 15 chemical fungicides was investigated under laboratory conditions. The effect of direct IJ exposure to fungicides for 24 h was tested in a petri dish at 15, 20, and 25 ºC. The results showed that the compatibility of *S. feltiae* with azoxystrobin was high, and similar findings were obtained for *S. carpocapsae* (strain C67) and all of the tested fungicides, except for tebuconazole + spiroxamine + triadimenol, maneb, dinocap, and copper (II) hydroxide + metalaxil-M. Nematode *H. downesi* (strain 3173) suffered the highest mortality rate when infective juveniles were mixed with tebuconazole + spiroxamine + triadimenol. The integration of the aforementioned agents into a pest management program is also discussed.

Key words: Compatibility, fungicides, *Heterorhabditis downesi*, *Steinernema carpocapsae*, *Steinernema feltiae*, viability.

Rhabditid nematodes of the Steinernematidae and Heterorhabditidae family are lethal to a broad range of economically important insect pests (Journey and Ostlie, 2000). Entomopathogenic nematodes (EPN) are often applied to sites and ecosystems that routinely receive other inputs that may interact with nematodes, including chemical pesticides, fertilizers, and soil amendments (De Nardo and Grewal, 2003). To save time and money, it is often beneficial to determine if a pesticide can be tank-mixed or applied simultaneously with another pesticide. In addition, the compatibility of an agent with integrated pest management (IPM) and integrated production (IP) systems must be evaluated (Grewal, 2002).

EPN infective juveniles (IJ) can tolerate short-term exposure (2-24 h) to many chemical and biological insecticides, fungicides, herbicides, fertilizers, and growth regulators, which can be tank-mixed and applied together (Krishnayya and Grewal, 2002; De Nardo and Grewal, 2003). However, generalizations cannot be applied because the nematode's susceptibility depends on several factors, including the species, strain, agrochemical formulation and application dose (Grewal, 2002).

Biological control agents are used to control a wide range of foliar insect pests (Trdan *et al*., 2007; Laznik *et al*., 2010a). When applied under conducive conditions, nematodes can be as effective as chemical insecticides (Trdan *et al*., 2007; Laznik *et al*., 2010a). Nematode-fungicide combinations in tank mixes could offer a cost-effective alternative to foliar integrated pest management (IPM) systems. However, before an ecologically integrated approach to pest management involving nematode-fungicide combinations in tank mixes can be developed for foliar application, the compatibility of nematodes with new and routinely used fungicides must be established.

To increase our knowledge of the susceptibility of EPN species to agrochemicals (fungicides) and to explore the effect of their mechanism on the viability of these organisms, the aim of the present study was to select several commercial fungicides currently used in Slovenia for crop protection, evaluate their effects on the survival of IJ from native Slovenian strains of *Steinernema feltiae* (Filipjev) and *Steinernema carpocapsae* Weiser, a Hungarian strain of *Heterorhabditis downesi* Stock (Griffin & Burnell) and commercial ENTONEM® strains

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at different temperatures under laboratory conditions, and determine their suitability in IPM programs.

MATERIALS AND METHODS

Fungicides

In the present study, 15 commercial fungicides registered against different fungal pathogens in Slovenia were evaluated. The tested fungicides were Fosetyl: ethyl hydrogen phosphonate (3.7 g L^{-1}; 0.25% Aliette flash; Bayer CS), Boscalid: 2-chloro-*N*-(4′-chlorobiphenyl-2-yl) nicotinamide + Pyraclostrobin: methyl 2-[1-(4-chlorophenyl)pyrazol-3-yloxymethyl]-*N*-methoxycarbanilate) (0.8 g L^{-1}; 0.08% Bellis; manufacturer: BASF SE Germany; distributed by BASF Slovenia, d.o.o.), Fluquinconazole: 3-(2,4-dichlorophenyl)-6-fluoro-2-(1*H*-1,2,4-triazol-1-yl)quinazolin-4(3*H*)-one + Pyrimethanil: *N*-(4,6-dimethylpyrimidin-2-yl) aniline (1.5 mL L^{-1}; 0.1% Clarinet; manufacturer: BASF SE Germany; BASF Slovenia, d.o.o.), Copper (II) hydroxide (4 g L^{-1}; 0.4% Cuprablau-Z; manufacturer: Cinkarna Celje, d.d., Slovenia), Maneb: manganese ethylenebis(dithiocarbamate) (2.5 g L^{-1}; 0.2% Dithane M-45; manufacturer: Dow Agrosciences; distributed by Karsia, Dutovlje, d.o.o), Tebuconazole: (*RS*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol, Spiroxamine: 8-*tert*-butyl-1,4-dioxaspiro[4.5]decan-2-ylmethyl(ethyl)(propyl)amine + Triadimenol: (1*RS*,2*RS*;1*RS*,2*SR*)-1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol (0.4 mL L^{-1}; Falcon EC-460; manufacturer: Bayer CS; Bayer Cropscience d.o.o.), Folpet: *N*-(trichloromethylthio) phthalimide (150 mL L^{-1}; 0.15% Folpan 80 WDG; manufacturer: Makhteshim-Agan; Karsia, Dutovlje, d.o.o), Sulfur (6 g L^{-1}; 0.2% Pepelin; manufacturer: BASF SE Germany; Cinkarna Celje, d.d., Slovenia), Metiram: zinc ammoniate ethylenebis(dithiocarbamate) - poly(ethylenethiuram disulfide) (1.2 g L^{-1}; 0.12% Polyram DF; manufacturer: BASF SE Germany; distributed by BASF Slovenia, d.o.o.), Propamocarb: propyl 3-(dimethylamino)propylcarbamate (2.5 mL L^{-1}; 0.15% Previcur 607 SL; manufacturer: Bayer CS; Bayer Cropscience d.o.o.), Copper (II) hydroxide + Metalaxil-M: methyl *N*-(methoxyacetyl)-*N*-(2,6-xylyl)-D-alaninate (4 g L^{-1}; Ridomil Gold Plus 42.5 WP; manufacturer: Syngenta; Syngenta Agro, d.o.o.), Azoxystrobin: methyl (2*E*)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy] phenyl}-3-methoxyacrylate (1 mL L^{-1}; 0.075% Quadris; manufacturer: Syngenta; Syngenta Agro, d.o.o.), Dinocap: (*RS*)-2,6-dinitro-4-octylphenyl crotonates (0.4 mL L^{-1}; Sabithane; manufacturer: Dow Agrosciences; distributed by Karsia, Dutovlje, d.o.o), Maneb + Propamocarb: (4 mL L^{-1}; Tattoo; manufacturer: Bayer CS; Bayer Cropscience d.o.o.) and Fenhexamid: 2′,3′-dichloro-4′-hydroxy-1-methylcyclohexanecarboxanilide (2 mL L^{-1}; Teldor SC 500; manufacturer: Bayer CS; Bayer Cropscience d.o.o.).

Nematodes

All of the strains were reared using the last instar larvae of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) (Bedding and Akhurst, 1975). *Galleria mellonella* production was executed in a rearing chamber (type: RK-900 CH, producer: Kambič Laboratory equipment, Semič, Slovenia) at 28 ± 2 ºC and 60% relative humidity (RH), and using a 12:12 h photoperiod (Woodring and Kaya, 1988; Parra, 1998). Four strains were included in the experiment. The commercial preparation Entonem (a.i. *Steinernema feltiae*) was obtained from Koppert B.V. (Berkel en Rodenrijs, The Netherlands). All of the other strains were isolated from the soil. *Steinernema feltiae* C76 (Laznik *et al*., 2009a) and *Steinernema carpocapsae* C67 (Laznik *et al*., 2008) were isolated in Slovenia, while *Heterorhabditis downesi* 3173 was isolated in Hungary (Tóth, 2006). Two strains (C67 and 3173) were tested for the first time in the present study, while strain C76 was proven to be effective in a laboratory assay against the third-stage larvae of the common cockchafer (Laznik *et al*., 2009b). Only IJ less than 2 wk old were used in the present study (Gutiérrez *et al*., 2008). The IJ were stored at 4 ºC at a density of 3000 IJ mL^{-1}. The number of IJ in a previously prepared nematode suspension with an unknown concentration was determined by counting the number of nematodes in a droplet (5 µL × 5) and diluting (adding a tap water solution) or concentrating (reducing to an adequate volume with the assistance of centrifugation) the sample. In this manner, the selected concentrations of nematode suspensions were obtained (Laznik *et al*., 2010c). Prior to the compatibility experiment, nematode viability was determined, and only nematode stocks with > 95% survival rates were used (De Nardo and Grewal, 2003).

Compatibility test

All of the fungicides were tested at the highest recommended concentration. Stock solutions of the fungicides were prepared in water. To 30 mL of the fungicide at 120% of the recommended concentration, 6 mL of IJ at a density or 3000 IJ mL^{-1} was added. The addition of the IJ solution brought the concentration of the fungicide down to the recommended rate. Each plastic Petri dish (40 × 10 mm; Kemomed d.o.o., Slovenia) contained 5 mL of the given solution. IJ were counted in Petri dish arenas at each step (before they were added to the fungicide and immediately after mixing). Five replicates were used for each treatment, and the experiment was repeated three times. Water was used as a control treatment. The Petri dishes were placed in a rearing chamber (type: RK-900 CH, producer: Kambič Laboratory equipment, Semič, Slovenia) without light at temperatures of 15, 20, and 25 ºC and at 70% RH. The Petri dishes were placed in a chamber in blocks (each fungicide treatment represented one block). At all of the tested temperatures, the effect of evaporation

was negligible. The viability of IJ incubated in different chemicals was assessed after 24 h by removing 3 × 50-µL sub-samples from each of the replicated treatments. At least 100 nematodes were counted for each treatment and the control. Nematodes that did not move after prodding were considered dead.

Statistical analyses

Prior to analysis, all of the data were corrected for the mortality rate of the control group using Abbott's correction (Abbott, 1925). Mortality data were analyzed using multifactor ANOVAs in Statgraphics Plus for Windows 4.0 (Statistical Graphics Corp., Manugistics, Inc.), and different fungicides were applied as independent variables. Mean separation was performed using Tukey's procedure with $\alpha = 0.05$.

RESULTS AND DISCUSSION

Data analyses on the pooled results are presented in Table 1. Among the studied fungicides, the ($P \leq 0.05$) highest rate of IJ mortality for strain C76 and Entonem was obtained with maneb (from -89.4% to -100%) and tebuconazole, spiroxamine + triadimenol(-100%) (Table 2). Compared to the control treatment, significant differences in the IJ mortality rate of strain C76 were observed at 15 ºC with all of the studied fungicides, except azoxystrobin (-13.9%), which was not significantly different from the control group after 24 h. Compared to the control group, significant differences in the mortality rate of strain C76 IJ were observed at 20ºC and 25ºC with maneb, tebuconazole, spiroxamine + triadimenol, copper (II) hydroxide + metalaxil-M, fosetyl ethyl hydrogen phosphonate, fluquinconazole + pyrimethanil, and dinocap. For strain C76, azoxystrobin did not significantly influence the mortality rate of IJ at all of the studied temperatures (-13.9%; +5.6%; +26.9%) after 24 h (Table 2). Similar findings were obtained for Entonem and fluquinconazole + pyrimethanil (-28.1%; -18.8%; -27.8%) and maneb + propamocarb (-14.5%; -6.3%; +0.4%), which were not significantly different from the control treatment. Among all of the fungicides tested at 20 ºC, Entonem had the highest IJ mortality rate (Table 2).

Among the studied fungicides, the highest ($P \leq 0.05$) mortality rate of *S. carpocapsae* C67 IJ was obtained with tebuconazole, spiroxamine + triadimenol (-100%). Compared to the control group, significant differences in IJ mortality were obtained at 20 ºC after 24 h of incubation with tebuconazole, spiroxamine + triadimenol (-100%). Compared to the control groups, significant differences in IJ mortality rates were not observed at 15 and 20 ºC for fluquinconazole + pyrimethanil (-27.8%; -30.4%), propamocarb (-20.8%; +29.2%), azoxystrobin (-23.2%; +10.8%), and maneb + propamocarb (+2.13%; +34.3%). For *H. downesi* ($P \leq 0.05$), the highest mortality rate of IJ was obtained with tebuconazole, spiroxamine + triadimenol (-100%). Compared to the control group, significant differences among fungicides were not observed at 15 ºC after 24 h, except for tebuconazole, spiroxamine + triadimenol (-100%). At 20 ºC, tebuconazole, spiroxamine + triadimenol (-100%) and maneb (-52.8%) were significantly different from the control (Table 2). After 24 h, significant differences among almost all of the studied fungicides and the control group were observed at 25 ºC. However, similar results were not obtained for fenhexamid (-29.1%).

The results of our research show that maneb and tebuconazole, spiroxamine + triadimenol causes the highest mortality rate in *Steinernema feltiae* (as well as that in native strain C76 and commercial product Entonem), *S. carpocapsae* strain C67, and *Heterorhabditis downesi* strain 3173. In a similar study, maneb did not affect the IJ mortality rate of *Heterorhabditis bacteriophora* (Rovesti *et al*., 1988), and *S. feltiae* was tolerant to four tested fungicidally active ingredients, including fenhexamid (*N*-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide), kresoxim-methyl (methyl (*E*)-methoxyimino[α-(*o*-tolyloxy)-*o*-tolyl]acetate), and nuarimol ((*RS*)-2-chloro-4′-fluoro-α-(pyrimidin-5-yl) benzhydryl alcohol) (Radova, 2010). The results of the present study and those of previous investigations (Rovesti *et al*., 1988; Krishnayya and Grewal, 2002; De Nardo and Grewal, 2003), in which the compatibility of plant protection products with EPN was evaluated, revealed that compatibility is species-specific. The present findings on the compatibility of nematodes and fungicides also confirm the results of two similar preceding studies (Krishnayya and Grewal, 2002; De Nardo and Grewal, 2003), which demonstrated that azoxystrobin did not influence the mortality rate of *S. feltiae*. Similar results were also obtained in the present study. Namely, after 24 h, significant differences among strain C76 and the control treatment were not observed at all of the tested temperatures.

In our experiment, when the nematodes were mixed with fungicide, temperature had an important influence on IJ mortality rates. At higher temperatures, the mortality

Table 1. ANOVA results for the corrected mortality rates of infective juveniles of entomopathogenic nematodes (EPN).

	Infective juveniles		
Source	F	df	P
Treatment	153.23	15	< 0.0001*
EPN strain	251.33	3	< 0.0001*
Temperature	70.27	2	< 0.0001*
Replication in time	1.85	4	0.0703
Replication in space	0.28	2	0.7590
EPN strain × treatment	13.48	45	< 0.0001*
EPN strain × temperature	122.40	6	< 0.0001*
Treatment × temperature	10.39	30	< 0.0001*
EPN strain × temperature × treatment	7.11	90	< 0.0001*

*Source of variation significant at $\alpha = 0.05$.

Table 2. Percent change in the survival of different entomopathogenic nematodes (EPN) strains after incubation with 15 different fungicides at 15, 20, and 25 ºC for 24 h.

	Change in nematode survival after exposure to chemicals for various durations at different temperatures (%)											
	Steinernema feltiae strain C76			*S. feltiae* strain Entonem			*S. carpocapsae* strain C67			*Heterorhabditis downesi* strain 3173		
Treatments	15 ºC	20 ºC	25 ºC	15 ºC	20 ºC	25 ºC	15 ºC	20 ºC	25 ºC	15 ºC	20 ºC	25 ºC
Aliette flash[1]	-59.9b*	-20.8ab	-37.8bc	-46.3cd*	-35.5cd	-11.5a	-63.0def*	-22.3bcd	+7.9ab	+22.9a	-38.2de	-59.3bcde
Bellis[2]	-58.6b	+1.4a	+1.5ab	-33.7bc	-39.4cde	+1.2a	-38.5bcd	-22.9bcd	-48.7cdef	+17.2a	-24.5cde	-49.2bcde
Clarinet[3]	-68.2b	+2.8a	-38.4bc	-28.1abc	-18.8abc	-27.8a	-27.8ab	+63.3a	-58.9ef	+9.0a	-34.0de	-51.4bcde
Cuprablau-Z[4]	-65.1b	-8.4a	-2.1ab	-38.8bcd	-53.3def	-12.7a	-37.6bcd	-21.0bcd	-14.7abc	+9.0a	+3.8abcd	-69.6cdef
Dithane M-45[5]	-98.2cd	-98.6c	-92.8de	-100.0e	-98.6h	-99.6b	-89.6fg	-65.4de	-41.9cdef	-64.7bc	-52.8e	-72.7def
Falcon EC-460[6]	-100.0d	-100.0c	-100.0e	-100.0e	-100.0h	-100.0b	-100.0g	-100.0e	-100.0g	-100.0c*	-100.0f	-100.0f
Folpan 80 WDG[7]	-61.4b	-32.0ab	-41.0bc	-43.2cd	-45.6de	-9.1a	-35.2bcd	+2.5abcd	-24.5bcde	-7.4ab	+14.1abc	-60.5bcde
Pepelin[8]	-60.8b	-8.4a	-24.4b	-25.4abc	-64.5efg	-4.8a	-46.2bcde	-33.4bcd	-17.0abcd	+0.8ab	-16.1bcde	-68.4cde
Polyram DF[9]	-65.7b	-17.4ab	-28.8b	-39.7bcd	-58.5defg	-13.5a	-53.2cde	+17.6ab	+13.9a	+4.1a	-13.2bcde	-64.3bcde
Previcur 607 SL[10]	-72.8bcd	-7.6a	-7.3ab	-41.5bcd	-18.5abc	+0.0a	-20.8ab	+32.0ab	-47.6cdef	+22.9a	-20.3bcde	-44.8bcde
Ridomil Gold Plus 42.5 WP[11]	-77.2bcd	-68.0bc	-87.6cde	-66.9d	-79.1fgh	-98.0b	-74.3efg	-50.3cde	-50.2cdef	-22.9a	-33.5de	-67.0cde
Quadris[12]	-13.9a	+5.6a	+26.9a	-22.3abc	-32.7bcd	-14.7a	-23.2ab	-24.9bcd	-50.2cdef	+25.4a	-17.9bcde	-42.7bcd
Sabithane[13]	-70.7bc	-37.5ab	-47.2bcd	-34.3bc	-82.6gh	-9.1a	-49.6bcde	-66.0de	-75.1fg	+22.2a	+45.3a	-75.2ef
Tattoo[14]	-52.2b	+13.9a	-25.9b	-14.5ab	-6.3ab	+0.4a	+2.13a	-0.7abcd	-43.1cdef	+26.2a	-4.7bcd	-41.4bc
Teldor SC 500[15]	-59.3b	-2.8a	-13.5ab	-27.5abc	-38.0cde	-23.8a	-41.9bcd	+8.4abc	-52.8def	-16.4ab	+25.0ab	-34.2b
Control (water)	100.0a	100.0a	100.0ab	100.0a	100.0a	100.0a	100.0a	100.0abcd	100.0 ab	100.0ab	100.0abcd	100.0a

*Values were significantly different ($P \leq 0.05$) in Tukey's multiple range tests. Small letters indicate that statistically significant differences were observed between the control treatment and fungicide treatments with the same EPN strain at the same temperature.

[1]Fosetyl: ethyl hydrogen phosphonate; [2]Boscalid: 2-chloro-*N*-(4′-chlorobiphenyl-2-yl) nicotinamide + Pyraclostrobin: methyl 2-[1-(4-chlorophenyl)pyrazol-3-yloxymethyl]-*N*-methoxycarbanilate); [3]Fluquinconazole: 3-(2,4-dichlorophenyl)-6-fluoro-2-(1*H*-1,2,4-triazol-1-yl)quinazolin-4(3*H*)-one + Pyrimethanil: *N*-(4,6-dimethylpyrimidin-2-yl)aniline; [4]Copper (II) hydroxide; [5]Maneb: manganese ethylenebis(dithiocarbamate); [6]Tebuconazole: (*RS*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol, Spiroxamine: 8-*tert*-butyl-1,4-dioxaspiro[4.5]decan-2-ylmethyl(ethyl)(propyl)amine + Triadimenol: (1*RS*,2*RS*;1*RS*,2*SR*)-1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol; [7]Folpet: *N*-(trichloromethylthio)phthalimide; [8]Sulfur; [9]Metiram: zinc ammoniate ethylenebis(dithiocarbamate) - poly(ethylenethiuram disulfide); [10]Propamocarb: propyl 3-(dimethylamino) propylcarbamate; [11]Copper (II) hydroxide + Metalaxil-M: methyl *N*-(methoxyacetyl)-*N*-(2,6-xylyl)-D-alaninate; [12]Azoxystrobin: methyl (2*E*)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy] phenyl}-3-methoxyacrylate; [13]Dinocap: (*RS*)-2,6-dinitro-4-octylphenyl crotonates; [14]Maneb + Propamocarb; [15]Fenhexamid: 2′,3′-dichloro-4′-hydroxy-1-methylcyclohexanecarboxanilide.

rates of IJ increased (the average number of living IJ at 15, 20 and 25 ºC was 2398.9 ± 128.3, 1947.4 ± 93.8, and 1720.2 ± 87.9, respectively). The activity of nematodes is highest (Laznik *et al*., 2010a) at temperatures ranging from 20 to 26 ºC, and their sensitivity, resistance, or tolerance to fungicides is related to their ability to withstand osmotic stresses; however, a sufficient amount of data on this interesting biological phenomenon has not yet been obtained (Thurston *et al*., 1994; Finnegan *et al*., 1999). Among the studied EPNs, the opposite pattern was observed for the *H. downesi* strain 3173 (the average number of living IJ at 15, 20, and 25 ºC was 1119.0 ± 171.9, 2491.4 ± 111.0 and 2199.9.2 ± 187.2, respectively), which was isolated in Hungary (Tóth, 2006) and is active at lower temperatures (12 ºC) (Lola-Luz *et al*., 2005). In our experiment, when combined with fungicide, this strain had lower rates of IJ mortality at higher temperatures.

The effect of pesticides on IJ movement (behavior) is difficult to evaluate because nematodes (especially *S. carpocapsae*) tend to remain quiescent and stay in a J-shaped position after treatment, but they can respond rapidly to mechanical stimuli (Rovesti and Deseo, 1990). The lack of precision in viability assays may lead to incongruence with infectivity data because features related to behavior such as movement inhibition, dispersion, and host attraction may be affected, as well as reproduction and development (Rovesti and Deseo, 1990; Ishibashi and Taki, 1993).

Based on the results of our research, EPN combined with fungicide treatment can offer better control management of an IPM program. IJ can be applied using nearly all commercially available ground or aerial spray equipment, including pressurized sprayers, mist blowers, and electrostatic sprayers (Georgis, 1990). EPN combined with fungicide treatment is a cost-effective alternative to chemical pest control because the effects of simultaneous application of fungicide and IJ on fungal and insect agents of plant diseases are similar and the proposed approach saves time and money spent in the control of pest organisms (Georgis, 1990). Azoxystrobin (Anand *et al*., 2008), propamocarb (Urban and Lebeda, 2007), and sulfur (Bassino *et al*., 1977) are well known for their efficacy against cucumber downy mildew (*Pseudoperonospora cubensis* [(Berk. & M.A. Curtis) Rostovzev]) and powdery mildew (*Erysiphe cichoracearum* DC). Several studies have shown that when EPN are correctly applied, they are effective on several types of cucumber pests, including western flower thrips (*Frankliniella occidentalis* Pergande) (Trdan *et al*., 2007) and the greenhouse whitefly (*Trialeurodes vaporariorum* [Westwood]) (Laznik *et al*., 2011). Synchronous application of fungicide and EPN for the control of the aforementioned pests is justifiable, especially under greenhouse conditions, where fungal and insect pests often occur together (Trdan *et al*., 2007; Laznik *et al*., 2011). Similar conclusions can also be drawn for metiram, copper (II) hydroxide, and maneb + propamocarb, which can be used to control potato blight (*Phytophthora infestans* [Mont.] de Bary), lettuce downy mildew (*Bremia lactucae* Regel), wheat leaf blotch (*Septoria tritici* Thüm.), and early tomato blight (*Alternaria solani* Sorauer) (Milus, 1994; Stepanović *et al*., 2009; Stevenson, 2009), along with several pest insects that often occur with the aforementioned fungi, such as the Colorado potato beetle (*Leptinotarsa decemlineata*

[Say]), cabbage armyworm (*Mamestra brassicae* [L.]), barley wireworm (*Agriotes fuscicollis* Miwa), cereal leaf beetle (*Oulema melanopus* [L.]), and tomato leafminer (*Tuta absoluta* [Povolny]), for which previous research has shown that EPN are effective biological control agents (Batalla-Carrera *et al*., 2010; Laznik *et al*., 2010a). Future implementation of the synchronous application of EPN and fungicides must be supported by field experiments because the results of laboratory experiments cannot be wholely extrapolated to environmental conditions.

CONCLUSIONS

According to the results of previous investigations and the current study, maneb and tebuconazole, spiroxamine + triadimenol are not compatible with *S. feltiae*, *S. carpocapsae* and *H. downesi*. In our experiment, when the nematodes were mixed with fungicide, temperature had an important influence on the mortality rates of IJ. Specifically at higher temperatures, the IJ mortality rates increased. Based on our research, EPN combined with fungicide treatment can offer better control management of an IPM program.

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Efecto de diferentes fungicidas en la viabilidad de nematodos entomopatógenos Steinernema feltiae (Filipjev), S. carpocapsae Weiser, and Heterorhabditis downesi Stock, Griffin & Burnell (Nematoda: Rhabditida) bajo condiciones de laboratorio. Para aumentar nuestro conocimiento sobre la susceptibilidad de especies de nematodos entomopatogénicos (EPN), se estudió la compatibilidad de los juveniles infectivos (IJ) de los EPN *Steinernema feltiae*, *S. carpocapsae*, y *Heterorhabditis downesi* con 15 fungicidas químicos bajo condiciones de laboratorio. El efecto de exposición directa de IJ a fungicidas por 24 h se evaluó en una placa Petri a 15, 20 y 25 °C. Los resultados mostraron que la compatibilidad de *S. feltiae* con azoxystrobin fue alta, y hallazgos similares se obtuvieron para *S. carpocapsae* (cepa C67) y todos los fungicidas probados, excepto para tebuconazole + spiroxamina + triadimenol, maneb, dinocap, y cobre (II) hidróxido + metalaxil-M. El nematodo *H. downesi* (cepa 3173) presentó la mayor tasa de mortalidad cuando los IJ fueron tratados con tebuconazole + spiroxamina + triadimenol. También se discute la integración de los agentes antes mencionados en un programa de manejo integral de plagas.

Palabras clave: Compatibilidad, fungicidas, viabilidad.

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