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ORIGINAL RESEARCH ARTICLE

Assessment of combined effects of some common agrochemicals and different entomopathogenic nematode species on the control of *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae) under controlled conditions

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Abstract

Entomopathogenic nematodes (EPNs) are an important component of integrated pest management (IPM) strategies; understanding their compatibility with other biocontrol options is of crucial importance in promoting their use. In this study, the toxicity of azadirachtin, sulfoxaflor, spindle oil (Mineral oil), and thyme oil was tested on the survival and pathogenicity of *Steinernema carpocapsae* (Weiser, 1955) (Rhabditida: Steinernematidae), *Steinernema bicornutum* sp. n. (Rhabditida: Steinernematidae), *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae), and *Heterorhabditis indica* (Poinar, Karunakar & David, 1992) under controlled conditions (25 ± 1 °C, 60 ± 10% RH). The toxicity of aforementioned agrochemicals to EPNs was evaluated in 12-well plates and the infective juveniles (IJs) of each EPN species (6000 IJs/50 µL ddh_2O) were exposed to the recommended field concentrations of agrochemicals. In the pathogenicity tests, the agrochemical and IJs solutions (3000 IJs/ml agrochemical) were applied to potato tubers in plastic containers which contained 20 adults of *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae). The survival of IJs and *P. citri* (Adults) was evaluated daily for three consecutive days after treatment. The results revealed that all tested EPN species were quite compatible with tested agrochemicals and showed survival of over 95% after 72 h of exposure to tested agrochemicals. EPNs and spindle oil combination induced the highest mortality of adult *P. citri*, and all EPN species caused mortalities over 90% at 72 h after treatment, except for *S. bicornotum*-spindle oil combination. Additive effects were observed in all combinations of EPNs with agrochemicals except for thyme oil. It appears, therefore, that EPNs applied in combination with azadirachtin, sulfoxaflor, and spindle oil have a great potential for successful control of *P. citri*. However, further studies are still required to determine the antagonistic or otherwise synergistic effects of tested chemicals on EPNs in controlling *P. citri* under field conditions.

Keywords Summer oil · Thyme oil · Sulfoxaflor · Beneficial nematodes · Compatibility · Integrated pest management (IPM)

Introduction

The Mediterranean region is one of the main suppliers of citrus fruits worldwide due to the suitability of the prevailing climatic conditions. However, the profitability of the cultivation greatly depends on proper management of citrus pests and diseases. Of the various insect pests infesting citrus orchards, the citrus mealybug, *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae), is considered one of the most problematic pests by citrus producers (Franco et al. 2004; Karacaoğlu and Satar 2017). Newly emerged nymphs and adult females of *P. citri* are able to

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feed on all above-ground plant parts, particularly on leaves and immature fruits which, subsequently, lead to deformation of leaves, general wilting, and early drop of the fruits (Urbaneja et al. 2020). Besides direct feeding damages, they also trigger the development of sooty mold that blocks sunlight and limits the photosynthetic capacity of the plants. Heavy infestations generally result in significant yield losses and a decrease in the commercial value of the fruits (Franco et al. 2004; Sirisena et al. 2013; Mahmood al., 2014; Mansour et al. 2017).

Recently, there has been a remarkable increase in the population of *P. citri* in the Mediterranean region of Türkiye, and considerable yield losses occurred due to these outbreaks (Karacaoğlu and Satar 2017; Özgökçe at al., 2021). Suppression of *P. citri* populations with insecticides is, however, quite challenging due to their small size and cryptic living habits, protective wax covering, and resistance to many insecticide groups (Rao et al. 2006; Venkatesan et al. 2016; Mruthunjayaswamy et al. 2019). Therefore, growers are forced to make multiple applications during the growing season, thereby exceeding the recommended application concentrations. Moreover, overuse of insecticides leads to pesticide residues on citrus fruits which generally give rise to the violation of legally established MRL (maximum residue level) limits (Sağlam and Masatcioğlu 2020). In addition, indiscriminate use of insecticides poses a serious threat to farmers, consumers, and has an adverse effect on the natural enemies of *P. citri*; which may be another reason behind the frequent outbreaks of *P. citri* (Franco et al. 2004; van Lexmond et al. 2015). Hence, it is essential to minimize the use of insecticides in the management of *P. citri* by combining chemicals with other control methods such as biological control.

Of the many attempts that have been made for a sustainable control of *P. citri*, entomopathogenic nematodes (EPNs) have shown great success in a number of studies (Demirci et al. 2011; van Niekerk and Malan 2012; Negrisoli et al. 2013; van Niekerk and Malan 2014; Najm et al., 2023). Infective juveniles (IJs) of EPNs are quite effective biological control agents of many economically important insect pests under favorable conditions; and some EPN species have active host-seeking abilities to locate and kill the target host (Shapiro-Ilan et al. 2017; Zhang et al. 2021). They are also compatible with many synthetic chemicals and biopesticides and can, therefore, be used concurrently to achieve a cost-effective and labor-saving control (Vashisth et al. 2013; Ulu et al. 2016). Most of the studies that have examined the effects of chemical insecticides and biopesticides on survival and pathogenicity of IJs have demonstrated that a combination of IJs and insecticides can be used successfully to control insect pests (Negrisoli et al. 2010; Laznik and Trdan 2014; Yüksel et al. 2019). However, the compatibility of chemical insecticides and biopesticides with EPNs may vary greatly according to EPN species/strains, chemicals, doses, and exposure time (Sabino et al. 2014; Khan et al. 2018). It is, therefore, crucial to determine the efficacy of those different chemical compounds on the survival and virulence of IJs under laboratory conditions before initiating field studies on their concomitant use against agricultural pests of economic importance including *P. citri*. In this regard, the present study aimed at evaluating the integrated use of different EPN species together with four commonly used agrochemicals (insecticide, biopesticide, thyme, and spindle oils) in the control of *P. citri*.

Table 1 List of entomopathogenic nematodes used in the experiments

Entomopathogenic nematode species	Strain	Habitat	Locality	GenBank Accession Number
Steinernema bicornutum	MGZ-4 S	Strawberry	Melikgazi-Kayseri	KX462912
Steinernema carpocapsae	E-76	Grassland	Melikgazi-Kayseri	KX462907
Heterorhabditis indica	216-H	Olive	Karatas-Kahramanmaraş	KP970842
Heterorhabditis bacteriaphora	FLH-4 H	Orchard	Felahiye-Kayseri	KX462939

Materials and methods

Source of nematodes and *Planococcus citri* and their rearing

Four EPN species [*Steinernema carpocapsae* (Weiser, 1955) (Rhabditida: Steinernematidae), *Steinernema bicornutum* sp. n. (Rhabditida: Steinernematidae), *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae), and *Heterorhabditis indica* (Poinar, Karunakar & David, 1992) (Rhabditida: Heterorhabditidae), identified by molecular and morphological methods in earlier studies (Canhilal et al. 2016, 2017), were included in the experiments (Table 1). Entomopathogenic nematodes were provided originally by Prof. Dr. Ramazan CANHİLAL of the Institute of Graduate School of Natural and Applied Sciences, Erciyes University. These EPNs were cultured in Petri dishes (9 cm diameter) on the last larval instar of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) in a growth chamber at 25 ± 2 °C, 60% RH. Aqueous suspension of IJs (1000 IJs/ml) was pipetted into the Petri dishes containing two filter papers at the bottom and sealed with parafilm. The Petri dishes were kept at 25 ± 2 °C, 60% RH in dark conditions, and dead *G. mellonella* larvae were relocated to modified White traps at 72 h after treatment (McMullen and Stock

2014). Emerged IJs were harvested, washed with sterile water, and transferred to culture flasks (250 ml). The flasks were kept horizontally at 14 °C and 60% RH until used in the experiments.

The laboratory culture of *P. citri* was initiated with the population obtained from the Department of Plant Protection, Faculty of Agriculture, Erciyes University. They were fed on potato sprouts (*Solanum tuberosum* L.) in plastic cages (60×60×60 cm) (Demirci et al. 2011) kept at 25 ± 2°C, 60 ± 10% RH, and 16-h light period conditions.

Galleria mellonella

Larvae of *G. mellonella* were originally obtained from the stock culture of Erciyes University (Department of Entomology) and kept in autoclaved glass jars (20 cm high and 10 cm in diameter) containing an artificial diet composed of wheat (400 g), corn flour (300 g), milk powder (200 g), yeast powder (100 g), honey (400 g), and glycerin (400 g). The jars were covered with plastic wire meshes to allow ventilation, and maintained in a growth chamber at 30 ± 2 °C, 60% RH (Metwally et al., 2012). After 4 weeks of incubation, the pupae were gently collected by hand and transferred to new jars containing filter papers. The emerged adults were provided with a honey-water (5%) solution daily as food. Eggs laid by the females on the filter papers were collected daily and put into new jars for the multiplication of insect population.

Agrochemicals

The four most commonly used agrochemicals in the control of *P. citri* (personal communication by representatives of both the Ministry of Agriculture and Forestry of Turkey and regional pesticide dealers), were included in the experiments (Table 2).

Table 2 Agrochemicals used in the experiment

Active ingredient	Trade name	Dose tested	Formulation	Company
Spindle oil (Mineral oil) 850 g/l	Protective Summer Oil	1250 mL/100 lt	SL	Koruma Klor Alkali Inc.
Sulfoxaflor 240 g/l	BREAKER™ 240	20 mL/100 lt	SC	Dow Agrosciences LTD.
Azadirachtin 10 g/l	NeemAzal T/S	1000 mL/100 lt	EC	Trifolio-M Gmbh Ltd.
Thyme oil (Pure) (*Thymus vulgaris* L.)	Thyme oil	3 mL/100 lt	EC	Doğan Baharatçılık Inc.

Experimental design

EPNs survival tests

The survival of IJs was assessed through a direct exposure of IJs to agrochemicals in 12-well plates. Aqueous suspension of IJs of each EPN species (6000 IJs/50 µL ddh_2O) was exposed to the recommended concentrations of agrochemicals in 2 mL of double distilled water (ddh_2O) (Table 2). Then, the well plates were covered with parafilm and put in an orbital shaker-incubator (25 ± 2 °C, 120 rpm) to prevent the settling down of agrochemicals at the bottom. The IJs were exposed to agrochemical solutions for 72 h and samples were taken at 24, 48, and 72 h after treatment. At each sampling time, 0.1 mL of suspension was taken from which randomly selected 100 IJs were examined under a stereo microscope (Zeiss, Germany), and the number of dead IJs was determined. Control treatments were performed only with ddh_2O. The experiments consisted of 4 replicates for each of the agrochemicals and the bioassays were conducted twice.

EPNs pathogenicity Bioassays

These bioassays were conducted in plastic containers (10×8×8 cm) containing one filter paper at the bottom. Twenty adult females of *P. citri* were transferred gently to the upper side of sprouted potatoes using a soft camel hair brush. Then, the potatoes were put into the plastic containers, and the mealybugs were allowed to feed on the tubers for 4 h before treatments. Two (2) mL of combined EPN-agrochemical solution (3000 IJs/ml) were applied to the potato tubers using a mini spray gun (Nozzle size 1 mm). Then, the containers were covered with a perforated lid to allow airflow, and maintained at 25 ± 1 °C, 60 ± 10% RH, and 12 h of light/dark photoperiods. The mortality of adult mealybugs was monitored daily for three consecutive days, and dead individuals were recorded. To confirm the nematode infection, dead adults were dissected to check whether nematodes were present. In the control treatments, ddh_2O water was used instead of agrochemicals, and the rest of the procedure was repeated as described above. Each treatment consisted of 4 plastic containers (4 replicates) and the bioassays were repeated twice.

Statistical analyses

Data from both experiments were subjected to normality tests and arcsine transformed. All statistical analyses were performed with SPSS software (22.0). Repeated measures MANOVA was performed to detect differences among treatments. Post hoc multiple comparisons were conducted

using Tukey's test ($P \leq 0.05$). EPN species, agrochemicals, and exposure time were considered explanatory variables. The response variables were the observed mortality of *P. citri* (Adults) and IJs of each EPN species at each exposure time.

Results

Survival of IJs

Significant differences, albeit small, were detected among separate and combined applications of EPNs with agrochemicals (Table 3). No mortality occurred in the control treatments for both bioassays (Survival and pathogenicity) during the experiments. Overall, the survival rates of IJs of the four EPN species exposed to the agrochemicals were higher; ranging from 92 to 99%, compared to the 100% survival rate recorded for unexposed IJs. The survival rates dropped, respectively, to 93.6 and 92.8% after 72 h for IJs of *S. carpocapsae* exposed to azadirachtin and spindle oil. Similarly, the survival of those of *S. bicornotum* was 94% at 72 h after treatment (Table 4).

Effect of agrochemicals on pathogenicity of EPNs

Mortality of *P. citri* was affected by all main factors (separate and combined applications of EPNs with agrochemicals) and their interactions (Table 5). A time-dependent increase in mortality rates was also observed for all combinations of IJs and agrochemicals. In general, the highest mortalities were obtained for all EPN-spindle oil combinations, except for *S. carpocapsae* - Sulfoxaflor combination 24 h after exposure. Thyme oil and its combinations with IJs of *H. bacteriophora* and *S. bicornotum* induced lower mortalities of *P. citri* at all exposure times compared to other combinations.

Table 3 Repeated measures analysis of variance (RM-ANOVA) parameters for the main factors and their associated interactions for the mortality of infective juveniles after 24, 48, and 72 h of exposure to different agrochemicals ($P \leq 0.05$)

Sources	Df*	*F*-value	*P*-value
Nematode species (N)	3	16.954	< 0.001
Agrochemicals (A)	4	100.415	< 0.001
N×A	12	12.519	< 0.001
Error-1	60		
Exposure time (t)	2	245.193	< 0.001
t×N	6	23.933	< 0.001
t×A	8	17.782	<0.001
t×N×C	24	10.126	< 0.001
Error-2	120		

*Degree of freedom

Table 5 Repeated measures analysis of variance (RM-ANOVA) parameters for the main factors and their associated interactions for the mortality of *P. citri* Risso 24, 48, and 72 h of exposure to different entomopathogenic nematodes in combination with agrochemicals

Sources	Df*	*F*-value	*P*-value
Nematode species (N)	3	9.070	< 0.001
Agrochemicals (A)	5	210.955	< 0.001
N×A	15	4.022	< 0.001
Error-1	72		
Exposure time (t)	2	1348.625	< 0.001
t×N	6	8.797	< 0.001
t×A	10	63.001	< 0.001
t×N×A	30	3.034	< 0.001
Error-2	144		

*Degree of freedom

Table 4 Survival rates of infective juveniles of entomopathogenic nematodes after 24, 48, and 72 h of exposure to different agrochemicals

EPN Species	Exposure time	Survival rates of Infective Juveniles (%±SE)				
		Control	Sulfoxaflor	Spindle oil	Thyme oil	Azadirachtin
S. carpocapsae	24 h	100.0A[a]a[b]	99.1 ± 0.4Aa	99.2 ± 0.5Aa	99.0 ± 0.6Aa	99.6 ± 0.5Aa
H. bacteriophora		100.0Aa	98.6 ± 0.4Ba	98.8 ± 0.3Ba	99.6 ± 0.2Ba	99.1 ± 0.4Ba
S. bicornotum		100.0Aa	98.7 ± 0.4Ba	98.2 ± 0.4Ba	96.8 ± 0.3Cb	98.3 ± 0.5Bab
H. indica		100.0Aa	100.0 ± 0.0Aa	99.2 ± 0.9Ba	98.4 ± 0.7BCa	97.6 ± 0.2Cb
S. carpocapsae	48 h	100.0Aa	98.5 ± 0.8Ba	98.6 ± 0.4Ba	98.8 ± 0.5Ba	99.2 ± 0.2Ba
H. bacteriophora		100.0Aa	98.2 ± 0.4Ba	97.4 ± 0.5Ca	99.2 ± 0.3Ba	98.8 ± 0.2Ba
H. indica		100.0Aa	98.6 ± 1.1Ba	98.0 ± 0.3Ba	97.5 ± 0.8Ba	97.5 ± 0.2Bab
S. bicornotum		100.0Aa	98.1 ± 0.3Ba	97.7 ± 0.1Ba	96.4 ± 0.3Cb	95.7 ± 0.8Cb
S. carpocapsae	72 h	100.0Aa	96.1 ± 1.5Ba	92.8 ± 1.9Cb	97.9 ± 0.1Ba	93.6 ± 0.4Cb
H. bacteriophora		100.0Aa	97.1 ± 1.8Ba	96.0 ± 0.2Ca	96.6 ± 0.8Ca	97.4 ± 1.3Ba
H. indica		100.0Aa	97.9 ± 1.4Ba	98.0 ± 0.3Ba	96.8 ± 0.4Ca	96.9 ± 0.2Ca
S. bicornotum		100.0Aa	97.6 ± 0.3Ba	97.4 ± 0.3Ba	94.0 ± 1.1Cb	95.4 ± 0.5Ca

*Each well received 6000 infective juveniles of entomopathogenic nematode species. [a]Capital letters indicate statistical differences among agrochemicals for each entomopathogenic nematode species. [b]Lowercase letters indicate statistical differences among entomopathogenic nematode species for each agrochemical (Tukey, $P \leq 0.05$)

Spindle oil combinations with *Heterorhabditis* species induced remarkable mortality on *P. citri* adults at 48 h after treatment compared to *Steinernema* species with over 97% mortality of adult *P. citri* recorded (Table 6). A similar trend was observed with Azadirachtin combinations to *H. bacteriophora* and *H. indica* at 72 h after treatment. The highest positive interaction with Sulfoxaflor was recorded for *S. carpacapsae* at 72 h after treatment with 80% mortality of adult *P. citri*. Thyme oil showed a positive effect only in conjunction with *H. indica* after 72 h of treatment, thereby inducing 72% mortality in adult *P. citri* (Table 6).

The activity of agrochemicals alone on *P. citri* revealed that spindle oil applications induced higher mortality of *P. citri* compared to the other tested agrochemicals. Spindle oil applications caused 56% mortality at 72 h after treatment followed by Sulfoxaflor with 35% mortality. Thyme oil caused the lowest mortality of *P. citri* at all exposure times which did not exceed 15% at 72 h after treatment (Table 7).

Discussion

This study investigated the compatibility of four EPN species with the main agrochemicals commonly used in the control of *P. citri*. The results revealed that IJs of EPNs exposed to tested agrochemicals showed survival of over 90% at 72 h after treatment. These results align with the findings of Krishnayyaand and Grewal (2002) who reported 84% IJ survival for *S. feltiae* after 120 h of exposure to Neem plant extracts. Similarly, Mahmoud et al. (2016) reported a 99% survival rate for IJs of *S. carpocapsae* and *H. bacteriophora* that were exposed to azadirachtin. Among the tested agrochemicals, sulfoxaflor showed the lowest toxicity to IJs. Sulfoxaflor is a potent insecticide that causes neurotoxic damage to targeted insects (Sparks et al. 2013). In a previous study, sulfoxaflor caused the lowest toxicity to the soil-dwelling roundworm *Caenorhabditis elegans* (Maupas, 1900), among tested insecticides; and did not induce significant lethality (Zhou et al. 2022), thereby confirming our findings. However, sensitivity of EPNs to chemicals may differ significantly depending on EPN species and strains. Indeed, Laznik and Trdan (2014) reported a high degree of variation in the susceptibility of different EPN species and strains to tested chemicals including azadirachtin. In another study, thyme oil at 1% concentration was highly toxic to *S. feltiae* and *H. bacteriophora* juveniles to which it caused 100% mortality (Barua et al. 2020), thereby contradicting our findings. These contradictions could be explained by the differences in the experimental design. For instance, Barua et al. (2020) evaluated the survival of EPNs at 1% thyme oil concentration which is quite high compared to the 10 µL/l used in the present study. In addition, compatibility

Table 6 The mortality rates (Mean ± SE) of *Planococcus citri* Risso (Hemiptera: Pseudococcidae) 24, 48, and 72 h of exposure to four EPN species in combination with agrochemicals

Treatments*	Mortality rates (%±SE)		
	24 h	48 h	72 h
Control (ddh_2O)	0.0A[a]d[b]	0.0Ac	0.0Ac
Sc	8.75 ± 4.8Cc	41.25 ± 8.5Bb	60 ± 10.0Ab
Sc + To	10 ± 4.1Cc	38.75 ± 8.5Bb	60 ± 7.1Ab
Sc + Aza	26.25 ± 4.8Cb	57.5 ± 6.5Bba	71.25 ± 9.4Aab
Sc + Sulf	52.5 ± 16.6Ba	65 ± 14.7Bba	80 ± 10.8Aab
Sc + So	46.25 ± 8.5Ca	77.5 ± 8.7Ba	92.5 ± 11.9Aa
Control (ddh_2O)	0.0Ac	0.0Ac	0.0Ac
Sb + To	1.25 ± 0.5Cc	21.25 ± 12.5Bb	43.75 ± 12.5Ab
Sb	8.75 ± 1.3Cb	28.75 ± 8.5Bb	45 ± 10.8Ab
Sb + Sulf	13.75 ± 4.8Cb	41.25 ± 4.8Bab	65 ± 7.1Aab
Sb + Aza	35 ± 7.1Ba	66.25 ± 8.5Aa	76.25 ± 8.5Aa
Sb + So	40 ± 14.7Ba	71.25 ± 8.5Aa	80 ± 5.8Aa
Control (ddh_2O)	0.0Ac	0.0Ad	0.0Ad
Hi	12.5 ± 1.9Cb	36.25 ± 11.8Bc	57.5 ± 16.6Ac
Hi + To	6.25 ± 4.8Cb	38.75 ± 8.5Bc	72.5 ± 8.7Ab
Hi + Sulf	22.5 ± 2.9Cab	65 ± 10.8Bb	75 ± 10.0Ab
Hi + Aza	21.25 ± 6.3Cab	66.25 ± 4.8Bb	82.5 ± 8.7Ab
Hi + So	45 ± 7.1Ba	97.5 ± 5.0Aa	98.75 ± 2.5Aa
Control (ddh_2O)	0.0Ac	0.0Ae	0.0Ae
Hb + To	2.5 ± 1.9Bc	16.25 ± 11.1Bd	46.25 ± 9.5Ad
Hb	20 ± 8.2Bb	42.5 ± 11.9Ac	61.25 ± 14.4Ac
Hb + Sulf	28.75 ± 8.5Cb	51.25 ± 11.8Bc	71.25 ± 8.5Abc
Hb + Aza	43.75 ± 14.9Bab	78.75 ± 6.3Ab	87.5 ± 5.0Ab
Hb + So	61.25 ± 10.3Ba	98.75 ± 2.5Aa	98.75 ± 2.5Aa

*Sc: *Steinernema carpocapsae*; Sb: *Steinernema bicornotum*; Hi: *Heterorhabditis indica*; Hb: *Heterorhabditis bacteriophora*. Aza: Azadirachtin; Sulf: Sulfoxaflor; So: Spindle oil; To: Thyme oil. Each treatment consisted of 20 adults of *Planacoccus citri* and was replicated four times. [a]Capital letters indicate statistical differences among exposure times for each treatment. [b]Lowercase letters indicate statistical differences among different treatments for each exposure time (Tukey, $P \leq 0.05$)

Table 7 The mortality rates (Mean ± SE) of *Planococcus citri* Risso (Hemiptera: Pseudococcidae) 24, 48, and 72 h of exposure to different agrochemicals

Treatments	Mortality rates (%±SE)*		
	Exposure time		
	24 h	48 h	72 h
Control	0.0b	0.0c	0.0c
Thyme oil	1.2 ± 0.5b	1.2 ± 0.5c	15.0 ± 10.8b
Azadirachtin	5.0 ± 2.7ab	15.0 ± 9.1b	30.0 ± 11.5ab
Sulfoxaflor	10.0 ± 4.0ab	26.2 ± 8.5a	35.0 ± 10.8ab
Spindle oil	21.2 ± 2.5a	38.7 ± 7.5a	56.2 ± 10.3a

*Each treatment consisted of 20 adults of *Planacoccus citri* and was replicated four times [a]Lowercase letters indicate statistical differences among different treatments for each exposure time (Tukey, $P \leq 0.05$)

of EPNs with agrochemicals could be a strain-specific characteristic, as suggested by Laznik and Trdan (2014). Morever, tolerance of nematodes to agrochemicals seems to vary according to species/strains, chemicals, exposure time, environmental conditions, and application concentrations (Wang et al. 2010; Kulkarni et al. 2017; Raheel et al. 2017; Özdemir et al. 2020). These factors, taken together, may explain the differences in the susceptibility of EPNs to agrochemicals.

Evaluation of the infectivity of EPNs in combination with agrochemicals revealed that the spindle oil combination of EPNs generally provided the highest efficacy on *P. citri*. This is probably due to the protective effect of spindle oil against desiccation. EPNs are, indeed, subject to desiccation that affects their viability and persistence, especially on exposed surfaces (Shapiro-Ilan et al. 2014; Ulu and Susurluk 2014). Spindle oil acts as a barrier to help preventing moisture loss, thus increasing the survival probability of EPNs post-treatment. Additionally, spindle oil may have provided a film layer for the IJs, thereby, enhancing their host-seeking ability and tolerance to environmental extremes. Similar to our findings, Aquino-Bolaños et al. (2019) evaluated the effectiveness of *S. carpocapsae*, *S. websteri*, and *H. bacteriophora* in oil emulsions on adult ticks and reported mortalities ranging between 80 and 100% 48 h after treatment. However, EPN species/isolates exhibit a significant adaptive genetic variation in their stress tolerance during unfavorable conditions, as suggested by Somvanshi et al. (2008), and Ulu and Susurluk (2014). Differences in adaptation capability and morphological characteristics may also have an impact on the pathogenicity of EPNs. In that respect, Timper and Kaya (1989) reported that *Heterorhabditis* species retained their J2 cuticles for a more extended period of time than *Steinernema* species; which might have provided advantages to *Heterorhabditis* species in our study to tolerate environmental extremes. Another variable that may have led to differences in the mortality rates of *P. citri* may be the different endosymbionts associated to EPNs. These bacteria play, indeed, a pivotal role in the pathogenicity of IJs by producing various secondary metabolites with insecticidal and immunosuppressant activities (Joyce et al. 2006; Hinchliffe et al. 2010). However, earlier studies revealed that the biochemical compounds released by endosymbionts of EPNs vary greatly among bacteria species/ strains and have varying degrees of toxicity against insect pests (Darsouei and Karimi 2018; Hasan et al. 2019; Vicente-Díez et al. 2021).

In the present study, all the tested agrochemicals exhibited low to moderate insecticidal activities against *P. citri*, with thyme oil showing the poorest efficacy (15%). Similar to our results, Szczepanik et al. (2012) reported poor acute toxicity of thyme oil on *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae), with mortality rates not exceeding 10% after 3 days. Raphael et al. (2011) reported a 43% mortality in *P. citri* after 8 days of exposure to seed kernel extract of *Azadirachta indica*. It is worth, however, to notice that phytochemicals are a complex mixture consisting of different biochemical compounds that can act as an endocrine disruptor, suffocant, and repellent (Odeyemi et al. 2008). To be effective, some of these compounds may need to be absorbed or ingested by the target pest. As for the present study, results revealed that the contact toxicity of thyme oil and azadirachtin remained limited probably due to the protective coating of *P. citri*. In addition, the presence of developed detoxication mechanisms in *P. citri* populations was documented in earlier studies (Venkatesan et al. 2016; Mruthunjayaswamy et al. 2019); and this might also have contributed to a tolerance of *P. citri* to azadirachtin and thyme oil.

Conclusion

Our results demonstrated that agrochemicals had a negligible effect on the survival of *S. bicornutum*, *S. carpocapsae*, *H. indica*, and *H. bacteriaphora*, and no significant mortalities occurred on the IJs of EPNs. The combinations of EPNs with agrochemicals provided better control against *P. citri* adults compared to separate applications of EPNs or agrochemicals, except for thyme oil. The highest mortality in *P. citri* was achieved when EPNs were applied in combination with spindle oil. Considering the obtained results, the combined application of EPNs with azadirachtin, sulfoxaflor, or spindle oil may enhance the biocontrol potential of EPNs against *P. citri* and hopefully other agricultural pests of economic importance. However, further studies are still needed under field conditions to confirm the efficacy of EPN species in combination with the tested agrochemicals.

Tables.

Abbreviations

EPNs Entomopathogenic nematodes
IJs Infective juveniles

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication This study does not contain any individual person's data.

Competing interests The authors have no competing interests.

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