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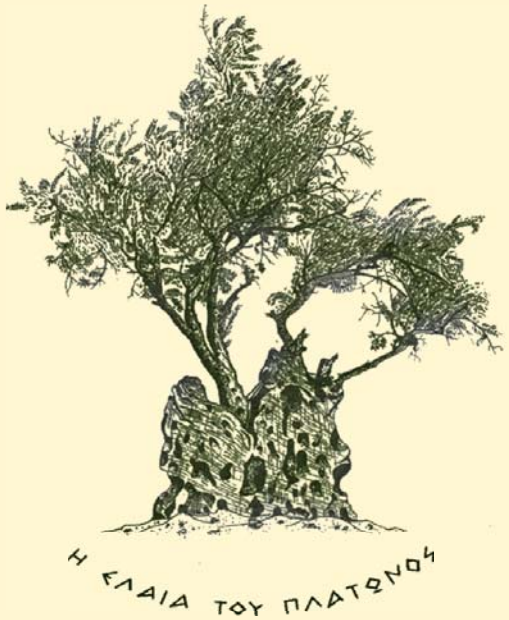

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SHORT COMMUNICATION

First report of *Fusarium proliferatum* causing stem and root rot on lucky bamboo (*Dracaena braunii*) in Iraq

A.A. Lahuf

Summary Lucky bamboo (*Dracaena braunii*) is a popular ornamental plant in Iraq. Individuals of this plant showing stem and root rot symptoms were observed during a survey conducted from November 2015 to February 2016 in several nurseries in Kerbala province, Iraq. Based on morphological characteristics and sequence analyses of the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA), the pathogen was identified as *Fusarium proliferatum*. This is the first report of stem and root rot caused by *F. proliferatum* on lucky bamboo (*D. braunii*) in Iraq.

Additional keywords: molecular identification, morphological characterization, pathogenicity

Lucky bamboo [*Dracaena braunii* (= *D. sanderiana*)] is an evergreen perennial ornamental plant of the Asparagaceae family, native to Cameroon in West Africa (Macedo and Barreto, 2016). Recently, it has become a popular ornamental houseplant in Iraq because of its beautiful appearance, low cost, its ability to grow under diverse indoor conditions and no experience required to take care of it.

During a survey conducted between November 2015 and February 2016 in ornamental nurseries in Kerbala province, Iraq, *D. braunii* plants showing stem and root rot symptoms were observed (Fig. 1A-D). Symptoms initially appeared on roots as water-soaked, red-brown lesions, becoming dark brown with time (Fig. 1B, D). Eventually, affected roots became completely rotten. On the lower part of the stem, a yellow discoloration was observed, tissues were soft and as the rot progressed, the diseased plants died Fig. 1A, C). The disease resulted in a significant loss of *D. braunii* plants in most of the nurseries examined. However, the pathogen causing this disease has not been previously investigated in Iraq. Thus, this study aims to isolate and identify the pathogen and assess its pathogenicity.

The symptomatic tissues of roots and stems were surface disinfected in 1% sodium hypochlorite for 2 min, rinsed three times with sterilized distilled water and dried with sterilized filter paper. Then the tissues were aseptically cut (0.5-1 cm long), placed onto 2% water agar (WA) medium and incubated in the dark at 25 ± 1°C for 3 days. Subsequently, a hyphal tip of each emerging fungal colony was sub-cultured on potato dextrose agar (PDA) medium supplemented with streptomycin sulphate (200 mg/l) and incubated in the dark at 25 ± 1°C for 7 days (Watanabe, 2010). Fungal colonies grew rapidly producing white aerial mycelia, occasionally with a violet pigmentation (Fig. 1E). The reverse colony color was pink to dark violet (Fig. 1F). Macroconidia were colourless and slightly curved with 3-5 septa and average size 33.4 × 3.2 µm. Microconidia were more than macroconidia, colourless, non-curved, occasionally in chains, with 0-1 septa and average size 8.2 × 3.1 µm. No chlamydospores were observed (Fig. 1G). These morphological features agree with the description of Leslie and Summerell (2006), except for the septation of the microconidia (0-septate according to Leslie and Summerell, 2006). However, the number of septa found in the present study are in line with

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the description of microconidia provided by Ichikawa, and Aoki (2000), Zhang *et. al.* (2013) and Kim *et. al.* (2016). Based on these morphological characteristics, the fungus was putatively identified as *Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg. To fulfil Koch's postulates, the pathogenicity of the isolated fungus was tested on 20 healthy lucky bamboo plants growing in 0.5 L containers filled with the commercial nutrition solution (AgroFiro®, Aljoud Company, Iraq). Fifteen plants were inoculated by adding directly to the nutrient solution five mycelium plugs (each 0.5 cm in diameter) cut from a 7-day old colony of *F. proliferatum* grown on PDA medium. The same number of plugs of un-inoculated PDA was added to the nutrient solution of the remaining five lucky bamboo plants, which were used as controls. All plants were incubated in a growth cabinet at 25 ± 2°C with 12-h photoperiod and 70% humidity. After 21 days, stem and root rot symptoms identical to those observed in the nurseries appeared on 13 out of the 15 inoculated plants. The control plants were symptomless. The fungal pathogen was re-isolated from the symptomatic plant tissues and showed the same morphological characteristics as described above.

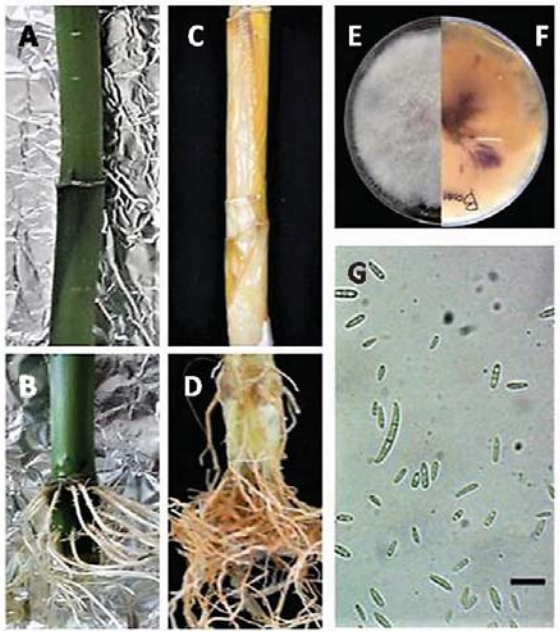

Figure 1. Symptoms of stem and root rot on *Dracaena braunii* plants, and cultural and morphological characteristics of the causal agent, *Fusarium proliferatum*. Stem (A) and roots (B) of a healthy *D. braunii* plant; rot symptoms on stem (C) and roots (D) of *D. braunii* plant infected by *F. proliferatum*; (E)-(F): colony of *F. proliferatum* on PDA medium (E: top surface and F: lower surface); (G): micro- and macroconidia of *F. proliferatum*; bar in (G) = 10 µm.

To confirm the initial morphological identification, the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) from the isolated fungus was sequenced. Genomic DNA of *F. proliferatum* was extracted from pure cultures using a DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's instructions. The universal primer pair ITS1/ITS4 was used to amplify the entire ITS region by PCR (White *et al*., 1990). The 679 bp amplicon was sequenced (Macrogen, Korea; http://www.macrogen.com/en/main/index.php) using the same primers used for the PCR amplification. The sequence was deposited into the GenBank database and was identified with the accession number MF099864.1. Subsequently, BLAST analysis of the isolate sequence showed >99% identity with several known sequences of *F. proliferatum* species. Phylogenetic analysis was performed using MEGA 7, utilizing the neighbor-joining technique (Tamura *et al*., 2013). This analysis showed that the ITS sequence of the isolate MF099864.1 was grouped in a clade comprising reference isolates of *F. proliferatum*. The out-group isolates were those of *Fusarium oxysporum* (accession No: EU326203.1), *F. camptoceras* (accession No: KU055634.1) and *F. solani* (accession No: L36632.1, L36634.1, AY097316.1, AY097317.1 and AY097318.1) (Fig. 2). Thus, these results support the preliminary morphological identification of the fungus as *F. proliferatum* (Leslie and Summerell, 2006; Zhang *et. al*., 2013; Aoki *et al*., 2014).

Numerous fungal pathogens are known to affect *Dracaena* spp. worldwide. For example, *Colletotrichum dracaenophilum* was reported to cause stem rot on *D. braunii* (syn. *D. sanderiana*) in Bulgaria, USA, Egypt and Brazil (Bobev *et al.,* 2008; Sharma *et al.,* 2014; Macedo and Barreto, 2016; Morsy and Elshahawy, 2016). In Iran, *Fusarium solani* was

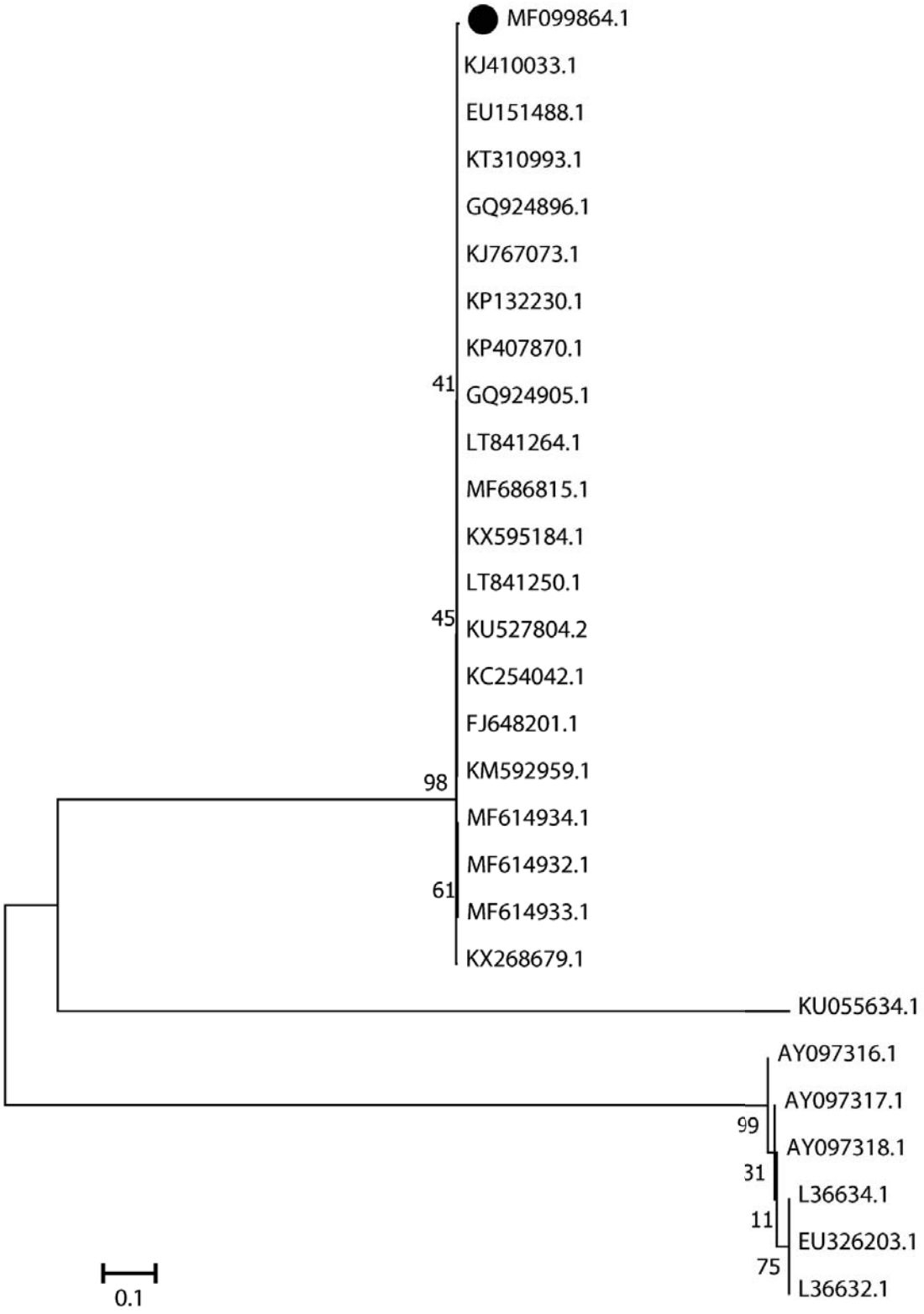

Figure 2. Phylogenetic tree constructed using ITS-rDNA sequences, presenting 21 known *Fusarium proliferatum* strains obtained from GenBank database, including that isolated in the present study from *Dracaena braunii* plants (MF099864.1; indicated with a black dot). Phylogenetic distances were calculated using the neighbor-joining method. Numbers above the branches refer to bootstrap values. *Fusarium oxysporum* (EU326203.1), *F. camptoceras* (KU055634.1) and *F. solani* (L36632.1, L36634.1, AY097316.1, AY097317.1 and AY097318.1) were the out-group species.

identified as causing stem rot disease on *D. sanderiana* (Abedi-Tizaki *et al.,* 2016). On the other hand, *F. proliferatum* is a devastating pathogen infecting a wide range of plant species throughout the world causing stem, crown and root rot as well as leaf proliferosis. In the USA and Canada, *F. proliferatum* was identified to cause root rot on *Glycine max* (soybean) (Arias *et al*., 2011; Chang *et al*., 2015). It was also reported on *Asparagus officinalis* (asparagus) causing crown and root rot in the USA and Turkey (Elmer, 1990; Özer *et al.,* 2011). In Argentina, *F. proliferatum* is described as a new pathogen causing root rot on *Vaccinium corymbosum* (blueberry) (Pérez *et al.,* 2011). In Malaysia, it was found associated with a stem rot disease of *Hylocereus polyrhizus* (Hawa *et al.,* 2013). In China, it was recorded causing root rot of *Medicago sativa* (alfalfa) and *Codonopsis lanceolata* (Cong *et al*., 2016; Gao *et al*., 2017). In Egypt, *F. proliferatum* var. *minus* was identified as the causal agent of leaf proliferosis disease of *D. sanderiana* (Wagih *et al*., 1989). To the best of my knowledge, this is the first report of *F. proliferatum* affecting *D. braunii* in Iraq.

The author is thankful to Dr Craig Simpson who works at The James Hutton Institute (UK) for revision of this paper.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη αναφορά του μύκητα *Fusarium proliferatum* ως αιτίου της σήψης στελέχους και ριζών σε φυτά *Dracaena braunii* (lucky bamboo) στο Ιράκ

A.A. Lahuf

Περίληψη Το *Dracaena braunii* (lucky bamboo) είναι ένα δημοφιλές καλλωπιστικό φυτό στο Ιράκ. Φυτά του συγκεκριμένου είδους, που εμφάνιζαν συμπτώματα σήψης του στελέχους και των ριζών, εντοπίστηκαν κατά τη διάρκεια επισκόπησης που διενεργήθηκε την περίοδο Νοέμβριος 2015-Φεβρουάριος 2016 σε αρκετά φυτώρια της επαρχίας Kerbala του Ιράκ. Με βάση τα μορφολογικά χαρακτηριστικά και τις αναλύσεις αλληλουχίας της περιοχής του εσωτερικού μεταγραφόμενου διαχωριστή (Internal Transcribed Spacer, ITS) του ριβοσωμικού DNA (rDNA), το παθογόνο ταυτοποιήθηκε ως ο μύκητας *Fusarium proliferatum*. Αυτή είναι η πρώτη αναφορά σήψης στελέχους και ριζών φυτών *D. braunii* από το μύκητα *F. proliferatum* στο Ιράκ.

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Resistance of ten common medicinal plants to the root-knot nematode *Meloidogyne javanica*

S. Ansari[1], H. Charehgani[1*] and R. Ghaderi[2]

Summary A preliminary survey indicated that the root-knot nematode *Meloidogyne javanica* is widely distributed in the rhizosphere of medicinal plants in Boyer-Ahmad region (Iran). Host suitability of ten species of medicinal plants to *M. javanica* was examined in a pot experiment under controlled greenhouse conditions: alkakengy (*Physalis alkekengi* L.), chamomile (*Matricaria chamomilla* L.), English plantain (*Plantago lanceolata* L.), fennel (*Foeniculum vulgare* Mill.), garden anchusa (*Anchusa italica* Retz.), horehound (*Marrubium vulgare* L.), lovage (*Levisticum officinale* L.), sorrel (*Rumex acetosella* L.), thistle (*Echinops adenocaulos* Boiss.) and woundwort (*Stachys pilifera* Benth.). According to the scheme of Canto-Saenz, seven species, namely garden anchusa, fennel, horehound, alkakengy, English plantain, woundwort and sorrel can be considered susceptible hosts with gall index (GI) > 2 and reproduction factor (RF) > 1, and thistle, lovage and chamomile, can be considered as hyper-susceptible with GI > 2 and RF ≤ 1.

Additional keywords: gall Index, hyper-susceptible, reproduction factor, susceptible

Introduction

Root exudates of plants contain chemical compounds which attract nematodes to the root or result in repulse, motility inhibition or even their death (Curtis *et al*., 2009). For example, chlorogenic acid which is subsequently oxidized by the action of host or nematode polyphenol oxidase might inhibit nematode activity and prevent root-knot nematode larvae from penetrating the endodermis into tissues suitable for giant cell production (Hung and Rohde, 1973). Three alkaloids namely sanguinarine, chelerytherine and allocryptopine have shown strong nematicidal activity (Wang *et al*., 2012). In addition, the phenolic acid compounds are potentially involved in resistance or tolerance of tall fescue (*Festuca* sp.) (Poaceae) to *Pratylenchus scribneri* (Pratylenchidae) (Bacetty *et al*., 2009).

Susceptibility of medicinal plants to parasitic nematodes vary between the species, from susceptible (Chinappen *et al.,* 1988; Rhoades, 1988; Mustika, 1992) to resistant (Mukhopadhyaya *et al.,* 1980; Tanda *et al.,* 1989; Haseeb and Butool, 1990; Haroon and Huettel, 1991) (Table 1). Studies by Sivakumar and Vadivelu (1997) on 46 medicinal and aromatic plants showed that *Meloidogyne hapla* (Heteroderidae) was the predominant nematode species followed by *Helicotylenchus indicus* (Hoplolaimidae), *Pratylenchus coffeae* (Pratylenchidae), *Tylenchorhynchus martini* (Belonolaimidae), *Xiphinema americanum* (Longidoridae), *Scutellonema conicephalum* (Hoplolaimidae) and *Hemicriconemoides mangiferae* (Criconematidae). Root-knot nematodes (*Meloidogyne* spp.) are serious pests of medicinal and ornamental plants (Haseeb *et al.,* 1984), which, in high population density can affect the quantity and quality of production (Haseeb *et al.,* 1996).

The objective of the present study was to determine the susceptibility of ten common medicinal plants as hosts to the root-knot nematode *Meloidogyne javanica* (Heteroderidae) under greenhouse conditions based on gall index (GA) and Reproduction Factor (RF), which are two important measures of nematode infestation (Sasser *et*

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al., 1984) and selection of resistant sources of different plants to root-knot nematodes (Talwana *et al.,* 1997; Cervantes-Flores *et al.,* 2008; Marchese *et al.,* 2010; Mudiope *et al.,* 2012; Gomes *et al.,* 2015; Karuri *et al.,* 2017).

Materials and Methods

Preparation of nematode inoculum

Eggs of *M. javanica* were extracted from galled root of tomato (*Solanum lycopersicum* cv. Early-Urbana) using the sodium hypoclorite method (NaOCl) (Hussey and Barker, 1973). Infected roots collected from the greenhouse of Boyer-Ahmad region were chopped to 2-3 cm pieces and were shaken in the 0.5 % sodium hypochlorite (NaOCl) for 90 seconds and poured into a stack of two sieves, with a 75 µm aperture size at top followed by a 25 µm aperture size. Eggs retained on 25 µm aperture size sieve, which were washed quickly to remove all NaOCl and were counted under a stereomicroscope.

Pot experiments

The experiments were conducted under greenhouse conditions at 28 ± 4ºC under 16:8 h (light : dark) photoperiod. Seeds of *Physalis alkekengi* L. (alkakengy) (Solanaceae), *Matricaria chamomilla* L. (chamomile) (Asteraceae), *Rumex acetosella* L. (sorrel) (Polygonaceae), *Plantago lanceolata* L. (English plantain) (Plantaginaceae), *Foeniculum vulgare* Mill. (fennel) (Apiaceae), *Anchusa italica* Retz. (garden anchusa) (Boraginaceae), *Marrubium vulgare* L. (horehound) (Lamiaceae), *Levisticum officina-*

Table 1. Degree of resistance (DR) of medicinal plants to the root-knot nematodes *Meloidogyne javanica* and *Meloidogyne incognita* (Walker, 1995; Baida *et al*., 2011).

Medicinal plants	DR to *M. incognita* race 3 (Walker, 1995)	DR to *M. incognita* (Baida *et al*., 2011)	DR to *M. javanica* (Baida *et al*., 2011)
Anethum graveolens L.	H	H	H
Artemisia absinthium L.	H	-	-
Erurca vesicaria L.	H	-	-
Foeniculum vulgare Mill.	H	-	-
Hyssopus officinalis L.	H	H	H
Lavandula augustifolia Mill.	H	-	-
Nepeta cataria L	H	-	-
Ocimum basilicum L.	H	H	H
Salvia officinalis L.	H	-	-
Thymus vulgaris L.	H	H	H
Mikania glomerata Sprengel.	-	H	H
Pimpinella anisum L.	-	R	H
Coriandrum sativium L.	S	-	-
Matricaria recutita L.	S	S	S
Melissa officinalis L.	S	-	-
Mentha piperita L.	-	-	-
Origanum majorana L.	S	R	R
Origanum vulgare L.	S	-	-
Rosmarinus officinalis L.	S	-	-
Ruta graveolens L.	S	R	R
Satureja hortensis L.	S	-	-
Tanacetum vulgare L.	S	-	-
Tomato (control)	-	S	S
Mentha pulegium L.	-	R	R
Plectranthus barbatus Andr.	-	R	R
Commiphora myrrha (Nees) Engl.	-	R	R
Carpobrotus edulis (L.) N.E. Br	-	R	R
Plectranthus neochilus Schltr.	-	R	R

S = Susceptible; H = Hypersusceptible; R=Resistance

le L. Koch (lovage) (Apiaeae), *Echinops adenocaulos* Boiss. (thistle) (Asteraceae) and *Stachys pilifera* Benth. (woundwort) (Lamiaceae) were sown in plastic pots (13 cm diameter and 10 cm height) containing 1.5 kg steam-sterilized sandy loam soil. After 45 days, each plant was inoculated with 5000 eggs + second stage juveniles (J_2) of *M. javanica* as the initial population (Pi). Inoculation was done by pipetting the egg + J_2 suspension into 3 holes around the plant root system. The experiment was conducted in a completely randomized design with four replications. The plants were watered daily and were harvested 60 days after inoculation.

The roots were gently washed with tap water and number of eggs in one gram of root were counted according to the procedure developed by Hussey and Barker (1973). One gram of root was stained with acid fuchsine according to the procedure developed by Byrd *et al.* (1983). The total number of eggs, galls and egg-masses per plant root system was determined by multiplying with the root weight per plant. The number of second stage juveniles (J_2)/100 cm^3 of soil was counted after extraction using the modified Baermann pie-pan method (Coyne *et al.,* 2014) and the total number of nematodes in soil was computed by extrapolating the number in 100 cm^3 to the volume of soil (1.5 kg).

The final nematode population (Pf) per pot (the total number of nematodes per plant root and the number of J_2 in soil per pot) were computed and finally, the reproductive factor (RF) of nematode was calculated by dividing the Pf by Pi (5,000 eggs + J_2). Gall index (GI) was estimated on a scale of 0 to 5, where 0 = no galls; 1 = 1 to 2 galls; 2 = 3 to 10 galls; 3 = 11 to 30 galls; 4 = 31 to 100 galls; and 5 = more than 100 galls in the root system (Taylor and Sasser 1978). The degree of resistance of medicinal plant species was allocated according to the modified scheme of Canto-Saenz (Sasser *et al.,* 1984), which is based on GI and RF as follows: resistant (GI ≤ 2, RF ≤ 1); tolerant (GI ≤ 2, RF > 1); hypersusceptible (GI > 2, RF ≤ 1); susceptible (GI > 2, RF > 1).

Statistical analysis

The SAS system V9.1 (SAS Institute Inc., Cary, NC, USA) was used for statistical analyses. Statistical analyses were performed using a one-way analysis of variance ANOVA and the significant difference between means was determined by Duncan's multiple range test (DMRT) ($p < 0.1$).

Results and Discussion

Sorrel and horehound had significantly higher number of galls and egg-masses, than the other plant species ($p < 0.01$) (Table 2). The lowest number of galls and egg-masses were recorded in chamomile, garden anchusa and thistle, being significantly lower than those on other plants ($p < 0.01$). The number of eggs in the sorrel root system and the number of J_2 per pot of garden anchusa were significantly higher than those on the other tested plants. Reproduction factor ranged from 0.05 (in chamomile) to 39.61 (in sorrel) but there was no significant difference among the RFs of chamomile and thistle, lovage, woundwort, english plantain and chamomile ($p < 0.01$) (Tables 2 and 3). Therefore, according to our results in Table 3, the resistance of tested medicinal plants to infection by *M. javanica* can be ranked as follows, according to the Canto-Saenz's scheme (Sasser *et al.,* 1984): thistle (*E. adenocaulos*), lovage (*L. officinale*) and chamomile (*M. chamomilla*) are classified as hypersusceptible, showing significant damage (GI > 2) while the RF remains below 1. Garden anchusa (*A. italica*), fennel (*F. vulgare*), horehound (*M. vulgare*), alkakengy (*P. alkekengi*), english plantain (*P. lanceolata*), woundwort (*S. pilifera*) and sorrel (*R. acetosella*) are ranked as susceptible, with heavy galling (GI = 5) and high reproduction factors (RF > 2). Sorrel and horehound are the most susceptible hosts with high reproduction factors (RF = 39.61 and 20.08, respectively).

Our findings on chamomile, *M. chamomilla,* to *M. javanica* (hyper-susceptible host) were similar to those by Baida *et al.* (2011) on susceptibility of *Matricaria recutita* L., while

Table 2. Mean population indices of the root-knot nematode *Meloidogyne javanica* on ten species of medicinal plants, 60 days after inoculation under greenhouse conditions.

Medicinal plants	Number of J_2 in soil	Number of eggs/root	Number of egg-masses/root	Number of galls/root
Physalis alkekengi L.	9525 ± 35.18^{c}	19514 ± 356^{c}	148 ± 5.10^{cd}	161 ± 6.03^{de}
Matricaria chamomilla L.	56 ± 7.18^{f}	184 ± 2.73^{e}	14 ± 1.79^{e}	21 ± 1.64^{f}
Rumex acetosella L.	37958 ± 859^{b}	176719 ± 6948^{a}	786 ± 82.61^{a}	801 ± 82.85^{a}
Plantago lanceolata	3143 ± 42.65^{e}	9306 ± 59de	344 ± 5.67^{b}	365 ± 5.43^{b}
Foeniculum vulgare Mill.	6619 ± 35.91^{d}	5104 ± 94.58^{e}	258 ± 6.57^{bc}	287 ± 6.69^{bc}
Anchusa italica Retz.	72380 ± 244^{a}	241 ± 8.95^{e}	98 ± 2.81^{de}	109 ± 4.32^{def}
Marrubium vulgare L.	5670 ± 66.24^{d}	99109 ± 1901^{b}	688 ± 20.14^{a}	734 ± 27.78^{a}
Levisticum officinale L.	640 ± 36.51^{f}	3411 ± 64^{e}	156 ± 6.51^{cd}	165 ± 5.09^{de}
Echinops adenocaulos Boiss.	0 ± 0^{f}	364 ± 10.27^{e}	16 ± 3.20^{e}	63 ± 10.26^{ef}
Stachys pilifera Benth.	919 ± 35.9^{f}	15506 ± 487^{cd}	190 ± 7.55^{cd}	197 ± 8.12^{cd}

Values in the same column followed by the same letter(s) are not significantly different ($p < 0.01$) based on Duncan's multiple range test (DMRT). Values are means ± standard error.

Table 3. Designation of resistance of ten species or aromatic plants to the root-knot nematode *Meloidogyne javanica* based on reproduction factor and gall index.

Medicinal plants	Reproduction factor	Gall Index	Resistance
Physalis alkekengi L.	5.56 ± 0.08^{c}	5	S
Matricaria chamomilla L.	0.05 ± 0.002^{d}	3	H
Rumex acetosella L.	39.61 ± 2.92^{a}	5	S
Plantago lanceolata	2.44 ± 0.006^{cd}	5	S
Foeniculum vulgare Mill.	2.28 ± 0.03^{cd}	5	S
Anchusa italica Retz.	5.19 ± 0.018^{c}	5	S
Marrubium vulgare L.	20.08 ± 0.38^{b}	5	S
Levisticum officinale L.	0.30 ± 0.0042^{d}	5	H
Echinops adenocaulos Boiss.	0.07 ± 0.002^{d}	4	H
Stachys pilifera Benth.	3.16 ± 0.10^{cd}	5	S

Values in the same column followed by the same letter(s) are not significantly different ($p < 0.01$) based on Duncan's multiple range test (DMRT). Values are means ± standard error.
Resistance based on Sasser *et al.* (1984): S = Susceptible; H = Hypersusceptible

Walker (1995) considered chamomile a susceptible host. Fennel was found to be a susceptible host whereas according to Walker (1995) is hyper-susceptible to *M. incognita* race3.

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Αντοχή δέκα κοινών φαρμακευτικών φυτών στον κομβονηματώδη *Meloidogyne javanica*

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Περίληψη Προκαταρκτική επισκόπηση έδειξε ότι ο κομβονηματώδης *Meloidogyne javanic*a είναι ευρέως διαδομένος στη ριζόσφαιρα φαρμακευτικών φυτών στην περιοχή Boyer-Ahmad (Ιράν). Εξετάστηκε η καταλληλότητα δέκα ειδών φαρμακευτικών φυτών ως ξενιστές του *M. javanica* σε πείραμα υπό ελεγχόμενες συνθήκες θερμοκηπίου: φυσαλίς (*Physalis alkekengi* L.), χαμομήλι (*Matricaria chamomilla* L.), πεντάνευρο (*Plantago lanceolata* L.), μάραθο (*Foeniculum vulgare* Mill.), αγχούζα (*Anchusa italica* Retz.), σκυλόχορτο (*Marrubium vulgare* L.), λουίζα (*Levisticum officinale* L.), ξινάκι (*Rumex acetosella* L.), εχίνοπας (*Echinops adenocaulos* Boiss.), στάχυς (*Stachys pilifera* Benth.). Σύμφωνα με το πρωτόκολλο του Canto-Saenz, επτά από τα είδη, η αγχούζα, ο μάραθος, το σκυλόχορτο, η φυσαλίς, το πεντάνευρο, ο στάχυς και το ξινάκι, μπορούν να θεωρηθούν ευαίσθητοι ξενιστές με δείκτη παρουσίας κόμβων του νηματώδη στις ρίζες (gall index - GI) > 2 και συντελεστή αναπαραγωγής (RF) >1, ενώ ο εχίνοπας, η λουίζα και το χαμομήλι, μπορούν να θεωρηθούν υπερ-ευαίσθητοι με GI> 2 και RF ≤ 1.

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Biodiversity and population fluctuations of parasitoids of the white peach scale, *Pseudaulacaspis pentagona* (Targioni-Tozzetti) (Hemiptera: Diaspididae), in kiwifruit orchards in Northern Iran

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Summary The white peach scale, *Pseudaulacaspis pentagona* Targioni-Tozzetti (Hemiptera: Diaspididae), is one of the most important and destructive polyphagous pests of the Rosaceae family trees. Population fluctuations and biodiversity of the hymenopteran parasitoid species associated with the pest were studied in six kiwi orchards in Iran, during one-year period. Parasitoid species abundance, species diversity indices and evenness indices were calculated. Most of the parasitoid species were dominant or eudominant. Based on the alpha diversity indices, the Najarkola region had high diversity and the Kharatkola region had low diversity. The Paeendasteh region (based on Simpson's Diversity on Camargo evenness indices) and the Samnakola region (based on the modified Nee, and on Smith and Wilson evenness indices) were less uniform. Among the recorded parasitoids, *Encarsia berlesei* Howard (Hymenoptera: Aphelinidae), followed by *Aphytis proclia* Walker (Hymenoptera: Aphelinidae), had the highest population in all orchards.

Additional keywords: Pseudaulacaspis pentagona, parasitoids, diversity, evenness index, species abundance, species richness

Introduction

The white peach scale, *Pseudaulacaspis pentagona* (Targioni-Tozzetti) (Hemiptera: Diaspididae), is the most important pest of kiwi fruit trees in Iran and other countries (Miller and Davidson, 2005; Toorani, 2017), attacking branches and twigs. The scale is most often seen in large numbers on the bottom of stems. The scale feeds on plant sap, and infestation causes leaves to yellow or defoliation and branches to dry. Fruit size may be reduced and premature drop is likely. Heavy infestations can result in stunting and the death of branches and dieback (Ezzat and Nada, 1986).

The families Aphelinidae and Encyrtidae are the most successful groups of Chalcidoidea, Hymenoptera used in the biological control of pest scale insects (Guerrieri and Noyes, 2000). Regarding reports on parasitoids of the white peach scale, the solitary endoparasitoid *Encarsia berlesei* Howard (Hym.: Aphelinidae) is considered to be the most effective species among the white peach scale natural enemies (Collins and Whitecomb, 1975), whose origin, like the white peach scale, is East Asia. *Aphytis chrysomphali* (Mercet) (Hym.: Aphelinidae) has been reported on the white peach scale in apricot and cherry trees from Shanghai, China (Invasive Species Compendium, 2016). In Iran, another three parasitoid species have been recorded, *Aphytis proclia* Walker (Hym.: Aphelinidae) (Modarres Awal, 1997), *Ablerus perspeciosus* Girault (Hym.: Aphelinidae) (Jamalomidi *et al*., 2012) and *Teleterebratus perversus* Compere and Zinna (Hym.: Encyrtidae) (Toorani, 2017).

Protecting biodiversity of taxonomic groups for which there are no available data on their existence or role in the ecosystem, is an important subject (Gaston, 1991). Alpha (α) diversity is intra (within) - habitat diversity. The species diversity is the main level

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of alpha diversity in the sense of the number of existing species and their abundance (evenness) in a geographical region, which increases with increasing number of existing species.

The present study was carried out to provide information on the species composition, diversity, evenness and population fluctuations of the hymenopterous parasitoids associated with the white peach scale, *P. pentagona,* in kiwi orchards in Iran.

Materials and methods

Experimental set up and data collection

This study was carried out in the kiwi orchards of Qaemshahr city, Mazandaran province of Iran. The laboratory experiments were carried out in the laboratory of Entomology and Insectarium of the Faculty of Agriculture Sciences of Shahed University of Tehran. In order to study the population fluctuations of parasitoid species associated with the pest under natural conditions on kiwi trees, six orchards, which had a history of pest infestation in previous years and had not received pesticides, at Qaemshahr and Mollakola areas (36° 35′ 44.20′′ N, 52° 46′ 35.09′′ E and -10.23 m a.s.l); Borjekheyl (36° 36′ 3.43 N, 52° 46′ 23.94 E and -13.28 m a.s.l); Kharatkola (36° 33′ 1.07′′ N, 52° 50′ 12.08′′ E and -4.23 m a.s.l); Paeendasteh (36° 36′ 39.59′′ N, 52° 47′ 47.39′′ E and -11.63 m a.s.l); Samnakola (36° 32′ 51.40′′ N, 52° 48′ 34.03′′ E and -0.21 m a.s.l and Najarkola (36° 33′ 20.26 N, 52° 48′ 55.42 E and -3.99 m a.s.l) were selected for sampling.

Sampling started on April 30, 2015, and ended on April 29, 2016. The samples were collected biweekly until January 22, 2016, and then monthly. Ten trees were randomly selected and marked on each date. Four infested branches were cut to a length of 10 cm each and placed in plastic glasses (5 cm diameter and 10 cm height), closed with a net cloth. Emerged parasitoids were collected and stored in 75% ethanol. In addition, on some sampling dates, a large number of infested branches of kiwi trees, were placed in cardboard boxes (50×20×45 cm) bearing six test tubes on each side of the box. The boxes were kept under natural conditions and emerging parasitoids were collected in the test tubes at 10-day intervals and stored in alcohol. The collected specimens were primarily identified and then, were sent for confirmation of identification, to Dr Andrew Polaszek, Department of Life Sciences, Natural History Museum.

Estimation of parasitoid species composition, abundance, diversity, evenness and population fluctuations

The data from the aforementioned six areas were used to calculate species diversity during 2015-2016. After identifying and counting the captured specimens, the dominant structure of species composition was evaluated using the method of Headman (Weigmann, 1973). In this method, the species, which their abundances are more than 30% of the society are identified as eudominant species, 10-30% as dominant, 5-10% as subdominant, 1-5% as rare, and less than 1% as sub-rare species.

Species diversity and relative abundance of the parasitoid species of *P. pentagona* were calculated in the different ecosystems of the Mazandaran province, using the Ecological Methodology software version 7.2. Based on the number of individuals per parasitoid species in each region, indicators of species diversity (number of species in a community, diversity in a region) and species evenness (number of individuals for each species, abundance and proportion of individuals of each species) were calculated.

Alpha species diversity was estimated using the Indices Shannon-Wiener (Shannon and Weaver, 1949) as the most common indicator to measure biodiversity and sensitive to the abundance of rare species in the community, Simpson's (Simpson, 1949) as a sensitive index to changes in more abundant species, and Brillouin (Pielou, 1969) as most appropriate for cases in which data are related to limited collections.

To estimate species equitability or even-

ness, the Smith and Wilson (Smith and Wilson 1996), modified Nee (Nee *et al.* 1992), Simpson (Simpson 1949) and Camargo (Camargo 1992) indices were used.

Results

Parasitoid species composition

In total, six parasitoid species were recorded in the study areas i.e. *Encarsia perniciosi* Tower (Hymenoptera: Aphelinidae), *A. chrysomphali, E. berlesei, A. perspeciosus, A. proclia, T. perversus*. Results for the species composition in each region are shown in Table 1.

Parasitoid species abundance

Encarcia perniciosi and *A. chrysomphali* had the highest abundance percent at Samnakola and Najarkola orchards, while *E. berlesei* and *A. proclia* had the highest abundance percent at Mollakola orchard. The abundance of *E. berlesei* species at Kharatkola orchard was (83.66%). The abundance of *A. perspeciosus* was zero at Mollakola, Borjekheyl and Kharatkola orchards (Table 1). According to Headman's categorization on species dominance proportion in the society (Weigmann, 1973), most of the six collected were eudominant or dominant (Table 1).

Alpha diversity

According to all indices, the parasitoid species diversity was higher in all regions compared to the Kharatkola region (Figure 1).

Species evenness

Based on the results obtained from the Camargo and Simpson eveness indices (Figure 2), the Paeendasteh region was the least uniform whereas according to the modified Nee index and the Smith and Wilson index, the Samnakola region were the least uniform (Figure 2). Based on diversity studies, the more uniform the species diversity is, the more diversity exists in the environment.

Population fluctuation of parasitoids

Figure 3 shows that the population of the parasitoids of the white peach scale dif-

Table 1. Relative abundance (%) and dominance proportion of parasitoids of the white peach scale, *Pseudaulacaspis pentagona,* in kiwi orchards in six regions of northern Iran during 2015-2016.

Species	Samnakola	Najarkola	Mollakola	Borjekheyl	Kharatkola	Paeendasteh
Encarsia perniciosi	30.9 Eudominant (233)	27.09 Dominant (207)	6.68 Subdominant (49)	6.08 Subdominant (54)	0 Subrare (0)	2.55 Rare (9)
Aphytis chrysomphali	25.9 Dominant (196)	52.9 Dominant (176)	7.77 Subdominant (57)	22.07 Dominant (196)	0 Subrare (0)	36.07 Eudominant (127)
Encarsia berlesei	19.8 Dominant (149)	19.63 Dominant (150)	45.42 Eudominant (333)	31.64 Eudominant (281)	83.66 Eudominant (128)	47.72 Eudominant (168)
Ablerus perspeciosus	17.1 Dominant (129)	19.37 Dominant (148)	0 Subrare (0)	0 Subrare (0)	0 Subrare (0)	9.94 Subdominant (35)
Aphytis proclia	5.7 Subdominant (43)	8.24 Subdominant (63)	40.10 Eudominant (294)	40.2 Eudominant (357)	12.41 Dominant (19)	1.70 Rare (6)
Teleterebratus perversus	0.53 Subrare (4)	2.61 Rare (20)	0 Subrare (0)	0 Subrare (0)	3.92 Rare (6)	1.98 Rare (7)

Parasitoid species categorization by Headman (Weigmann, 1973) according to their dominance proportion in the society: eudominant (>30%), dominant (10-30%), subdominant (5-10%), rare (1-5%), sub-rare (< 1%).
Number in parentheses indicate the sample size.

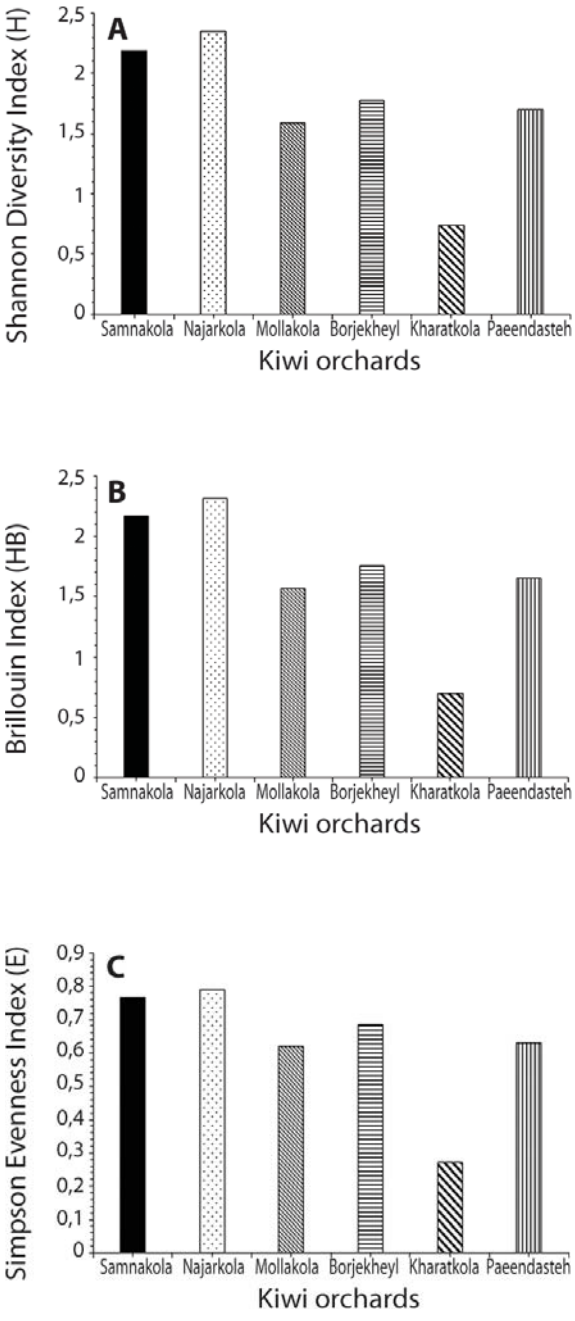


Figure 1. Alfa species diversity indices of parasitoids of the white peach scale, *Pseudaulacaspis pentagona*, in kiwi orchards in six regions of northern Iran during 2015-2016: A) Shannon Wiener diversity index, B) Brillouin diversity index, C) Simpson diversity index.

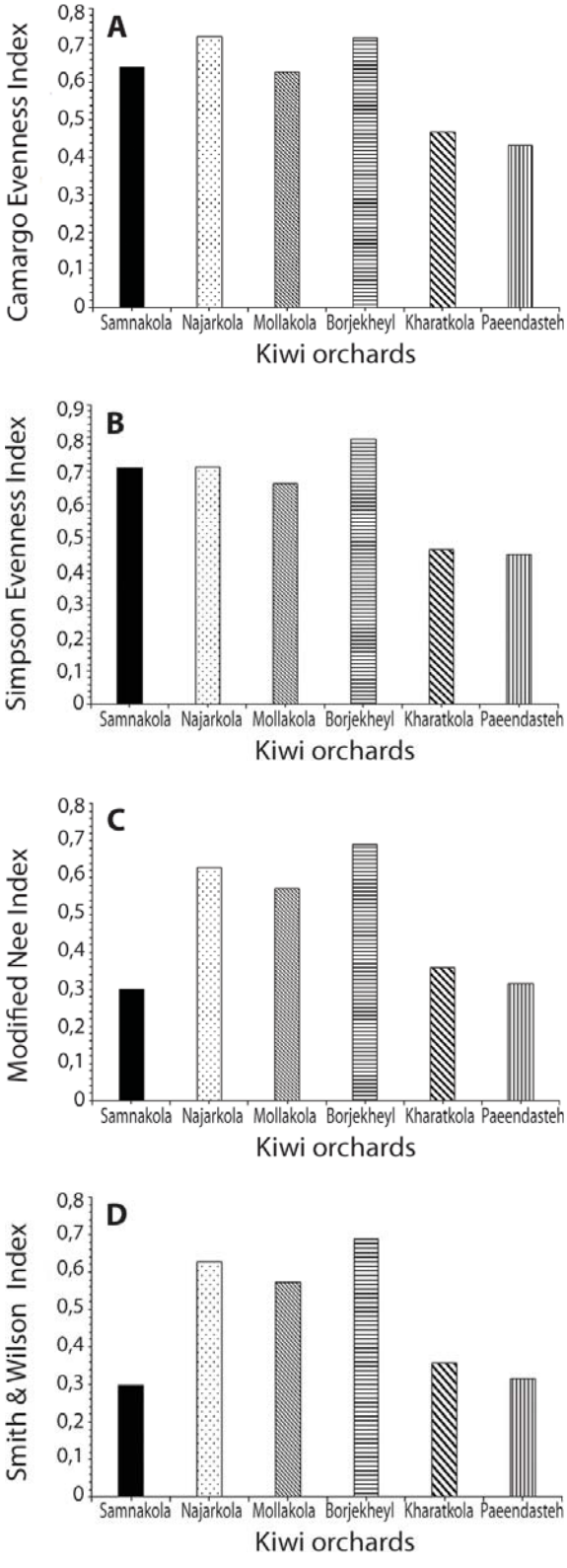
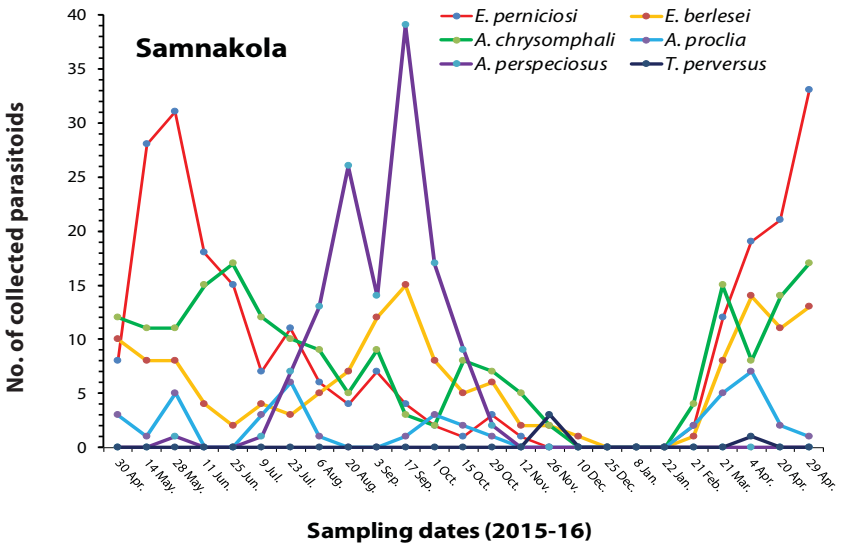
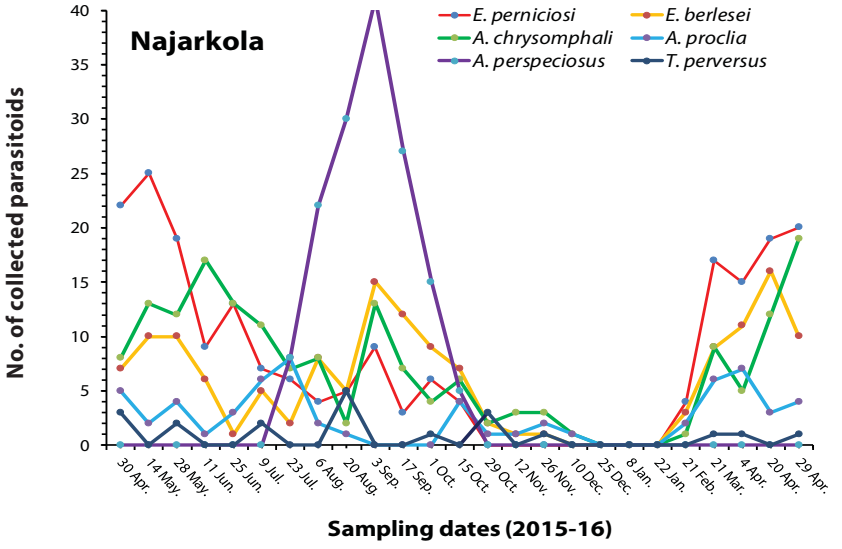
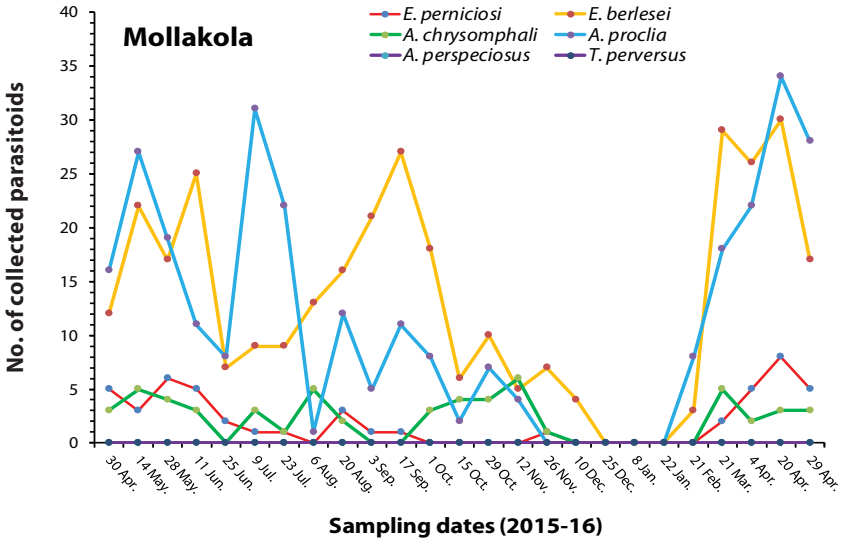


Figure 2. Species evenness indices of parasitoids of the white peach scale, *Pseudaulacaspis pentagona*, in kiwi orchards in six regions of northern Iran during 2015-2016: A) Camargo evenness index, B) Simpson evenness index, C) Modified Nee evenness index, D) Smith and Wilson evenness index.







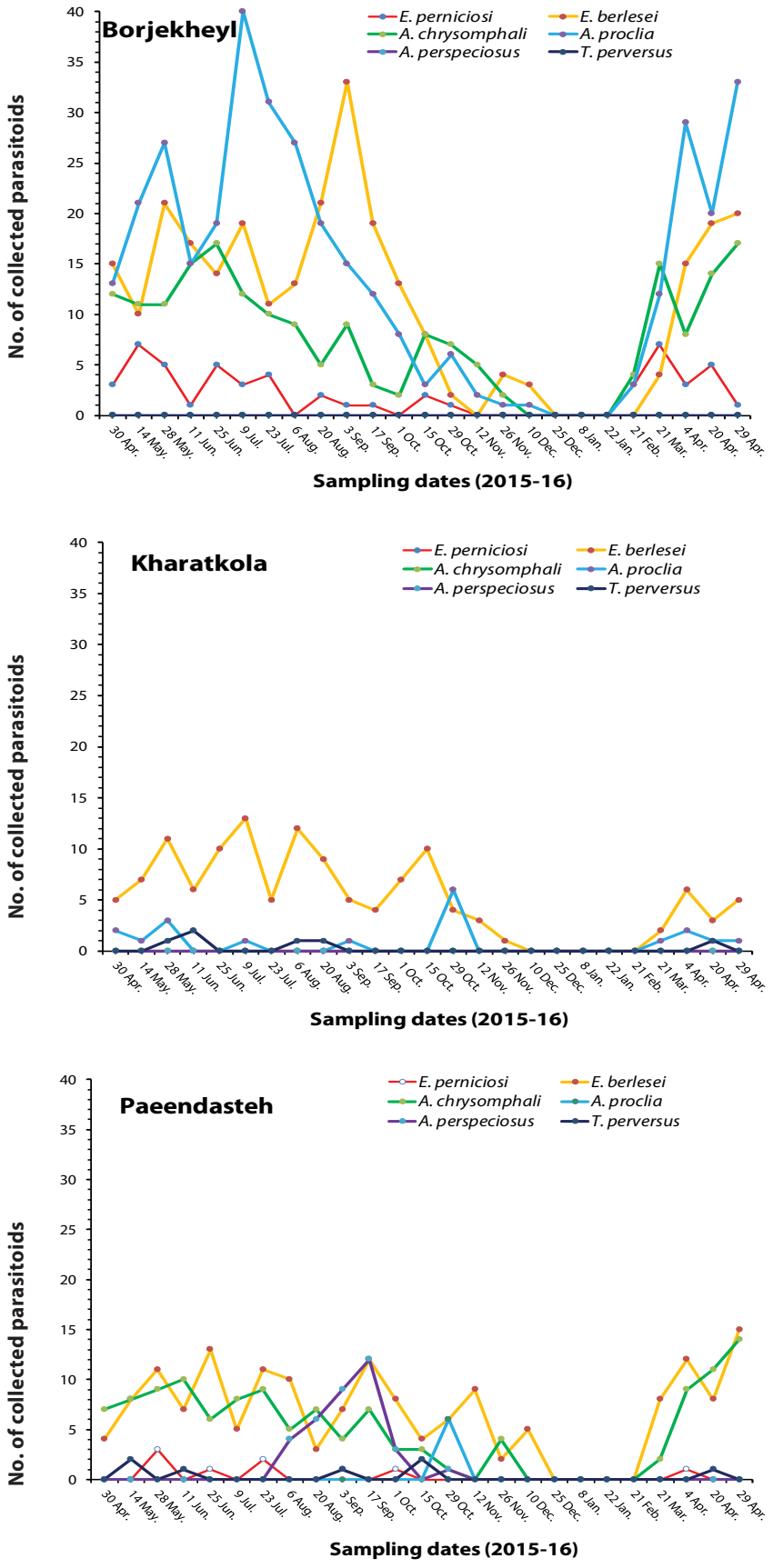


Figure 3. Population fluctuations of different species of parasitoids of the white peach scale, *Pseudaulacaspis pentagona*, in kiwi orchards of northern Iran during 2015-2016.

fers among the studied orchards as well as on different sampling dates, reaching the lowest (zero) at the end of December - beginning of January in all orchards due to coincidence with the winter colds and overwintering of these parasitoids.

In all orchards, *E. berlesei* (n= 1209) had the highest numbers followed by *A. proclia* (n= 782). These two species had significant ups and downs during sampling. *Ablerus perspeciosus* was observed in three orchards in the areas of Samankola, Najarkola and Paeendasteh, showing 1-2 peaks from August to October. *Encarsia perniciosi* and *A. chrysomphali* were collected from all the studied orchards, except for the one in Kharatkola, and were present from spring to autumn with their population reducing until reaching zero in the winter. *Teleterebratus perversus* was recorded in small numbers from spring to autumn at the orchards of the areas Najarkola, Kharatkola and Paeendasteh.

Discussion

To our knowledge this is the first record on the biodiversity and population fluctuation of the parasitoid species of the white peach scale in kiwi orchards in Iran. Five out of the total six species recorded in the samples are known to parasitize *P. pentagona* in Iran, i.e. *Encarsia berlesei* Howard (Hymenoptera: Aphelinidae) (the most effective), *Aphytis chrysomphali* (Hymenoptera: Aphelinidae) (Invasive Species Compendium, 2016) *Aphytis proclia* Walker (Hymenoptera: Aphelinidae) (Modarres Awal, 1997), *Ablerus perspeciosus* (Jamalomidi *et al*., 2012) and *Teleterebratus perversus* (Toorani, 2017). *Encarsia perniciosi* has not been reported on the white peach scale in Iran.

Encarsia berlesei was the most abundant of all parasitoid species in all regions of the study. Except of the wheat peach scale, it parasitizes another 10 species of the Diaspididae family, including *Aulacaspis cinnamomi* Newstead, *Chrysomphalus dictyospermi* Morgan, *Chrysomphalus obscurus* Lizer y Trelles, *Diaspis pentagona* Fargioni, *Melanaspis obscura* Comstock, *Nuculaspis abietis* Schrank, *Parlatoria pergandii* Comstock, *Pinnaspis minor* Maskell, *Pinnaspis strachani* Cooley and *Pinnaspis temporaria* Ferris (Natural History Museum, 2016). Other hosts of *A. chrysomphali* include various scale species in the families of Coccidae and Diaspididae (61 species) (Natural History Museum, 2016).

Records of other host scales of *E. perniciosi*, *A. proclia* and *A. perspeciosus* in Iran include: *E. perniciosi* has been reported on *Quadraspidiotus perniciosus* Comstock (Modarres Awal, 1997; Ghahari *et al*., 2011), *Aonidiella aurantii* Maskell, *Lepidosaphes ulmi* Linnaeus, *Parlatoria acalcarata* McKenzie and *Quadraspidiotus gigas* Ferris (Heraty *et al*., 2007); *A. proclia* has been recorded on *A. aurantii*, *Aonidiella orientalis* Newstead, *C. dictyospermi*, and *Parlatoria oleae* Colvee (Modarres *et al*., 1997); *A. perspeciosus* has been found on *Q. perniciosus* Comstock (Abd-Rabou and Ghahari 2005; Aliakbar Aghadokht *et al*., 2010) and *Aleurolobus barodensis* Maskell (Hemiptera: Aleyrodidae) (Jamalomidi *et al*., 2012).

Differences in the parasitoid species composition, richness and abundance among the studied areas could be attributed to several factors that can affect the presence of a parasitoid in the orchard, such as the type of vegetation around the orchard, the history of chemical applications in the orchard, the area and age of the trees, the population of the host pest, as well as environmental parameters (temperature, humidity, latitude and longitude, sea level altitude) (Lotfalizadeh *et al*., 2014; Habibi Badrabadi *et al*., 2017 and Iranmanesh *et al*., 2017). The richer species composition and abundance of parasitoid species of the white peach scale in Samnakola, compared to the other areas, could be related to the fact that the majority of orchards in this area are kiwi trees, dating for several years. The poorer species variety and number of parasitoids in Kharatkola could be associated to the presence of only one kiwi orchard and the young age of the trees, whereas rice is the dominant cultiva-

tion in the region.

Lower richness in the Kharatkola, Borjekheyl and Paeendasteh regions can be associated with the application of organophosphorus pesticides (such as chlorpyrifos and diazinon), whereas applications with mineral oil and botanical pesticides are related to higher richness in Samnakola, Najarkola and Mollakola (unpublished data). Nevertheless, Kyparissoudas (1987) showed that in orchards where chemical pesticides were applied, *E. perniciosi* wasps were not captured in pheromone traps of its host *Q. perniciosus*.

The population of parasitoid species of *P. pentagona* varied in the sampling regions. *Encarsia berlesei* had 4-6 population peaks on white peach scale in kiwi orchards in the present study. Bazrafshan *et al*. (2010) observed two peaks of the parasitism rate for the parasitoid on peach trees. Moreover, they showed that plant species has an effect on the rate of parasitism and the associated number of peaks. In our case, it is possible that the activity of parasitoids is favored by the micro-climate conditions in kiwifruit orchards (in comparison to peach orchards), where the pest is located on shoots in the shade.

According to Pedata *et al*. (1995), the population of *A. proclia* on white peach scale in a mulberry orchard in Campania, Italy, reached a peak in April, which is similar to the results of the present study. Seasonal abundance of *Aphytis* sp. had three larval and pupal peaks on white peach scale in peach trees in Dakahlia governorate, Egypt, in two successive seasons (2013-2014 and 2014-2015) (Halawa *et al*., 2015).

In the Hafez (1988) study, *A. chrysomphali* was found to be fairly abundant on *A. aurantii* in *Citrus sinensis* orchards in Alexandria, Egypt, with three peaks of activity in June, October and November.

Conclusions

In conclusion, results of the present study corroborate the existence of several hymenopterous parasitoid species of *P. pentagona* in kiwi fruit orchards in Iran. In general, *E. berlesei*, *A. chrysomphali* and *A. proclia* were abundant in most of the study regions. In view of the high prevalence of these species at the peak population dates, these results, together with the data on the parasitoid population changes over the crop season, can facilitate the designing of biological control programs against the white peach scale.

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Βιοποικιλότητα και πληθυσμιακές διακυμάνσεις παρασιτοειδών του κοκκοειδούς *Pseudaulacaspis pentagona* (Targioni-Tozzetti) (Hemiptera: Diaspididae) σε οπωρώνες ακτινιδίου στο Βόρειο Ιράν

A.H. Toorani, H. Abbasipour και L. Dehghan-Dehnavi

Περίληψη Το κοκκοειδές *Pseudaulacaspis pentagona* Targioni-Tozzetti (Hemiptera: Diaspididae) είναι ένας από τους πιο σημαντικούς και καταστρεπτικούς πολυφάγους εχθρούς των δέντρων της Οικογένειας Rosaceae. Οι διακυμάνσεις του πληθυσμού και η βιοποικιλότητα των υμενόπτερων παρασιτοειδών του κοκκοειδούς μελετήθηκαν σε έξι οπωρώνες ακτινιδίου στο Ιράν κατά τη διάρκεια ενός έτους. Εκτιμήθηκαν η σχετική αφθονία τους, δείκτες ποικιλότητας και δείκτες ισομέρειας. Τα περισσότερα από τα είδη των παρασιτοειδών ήταν κυρίαρχα (dominant) ή eudominant. Με βάση τους δείκτες ποικιλότητας, η περιοχή Najarkola είχε μεγάλη ποικιλομορφία και η περιοχή Kharatkola είχε μικρή ποικιλομορφία. Η περιοχή Paeendasteh (με βάση τους δείκτες ποικιλότητας Simpson και ισομέρειας Camargo) και η περιοχή Samnakola (με βάση τους δείκτες ισομέρειας, τροποποιημένος δείκτης Nee, και δείκτης Smith και Wilson) ήταν λιγότερο ομοιόμορφες. Μεταξύ των ειδών παρασιτοειδών, το *Encarsia berlesei* Howard (Hymenoptera: Aphelinidae) και το *Aphytis proclia* Walker (Hymenoptera: Aphelinidae) είχαν τον υψηλότερο πληθυσμό σε όλους τους οπωρώνες.

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SHORT COMMUNICATION

First record of *Chymomyza procnemoides* (Wheeler) (Diptera: Drosophilidae) for the Turkish fauna

E. Zengin

Summary This is the first record of *Chymomyza procnemoides* (Wheeler, 1952) (Diptera: Drosophilidae) in Turkey. The specimens were obtained from bottle bait traps on apple, pear and plum fruit trees at the Uşak province in 2017.

Additional keywords: Chymomyza procnemoides, Drosophilidae, new record, Uşak, Turkey

The genus *Chymomyza* is one of 75 genera belonging to the Family Drosophilidae, commonly known as vinegar or fruit flies. Sixty (60) species were identified in this genus, one of which is *Chymomyza procnemoides* (Markow and O' Grady, 2005; Yassin, 2013). This species is native to North America and was detected in the European continent for the first time in Hungary in 1990 (Band, 1994). Since then, no other records exist except from the Nearctic region.

Most members of *Chymomyza* species are attracted to damaged trees or cut wood that serve as feeding and breeding sites (Wheeler, 1952; Watabe, 1985; Band, 1995). Certain *Chymomyza* species have been recorded breeding in fruits such as apple, cherry and nut (Burla and Bachli, 1992; Burla, 1995).

In Turkey, 36 species belonging to six genera of Drosophilidae fauna have been reported (Koçak and Kemal, 2013). In this study, *C. procnemoides* was recorded at Bağkonak village (38°44'47"N, 29°46' 45" E, 920 m) in the district of Banaz, Turkey. It was detected in bottle bait traps for the first time at the end of September (23.9.2017) and later on early October (7.10.2017).

During the study, trapping was performed in Banaz, Uşak province between April and December in 2017. The traps were randomly hung on apple, pear and plum trees in mixed fruit orchards. They consisted of a plastic bottle of 500 ml containing 100 ml vinegar and bore 10-15 holes of 3 mm diameter at the top area to enable insect entrance. They were hung at a height of 1-1.5 m above the ground. The specimens obtained from the traps were preserved in 75% alcohol for identification. Among the Drosophilidae specimens, two female individuals, captured on different dates, were found to belong to the genus *Chymomyza* (Wheeler, 1952). The species was identified as *C. procnemoides* by Dr Paul Beuk (Maastricht Natural History Museum, Maastricht, Netherlands).

The most important features distinguishing *C. procnemoides* from other *Chymomyza* species include black fore femora, tibiae and metatarsi and inner edge of fore femora with a row of 6-10 short spines which is not as long as tibial diameter (Wheeler, 1952). *Chymomyza procnemoides* has black fore metatarsus, similar to *Chymomyza procnemis* Williston (Diptera: Drosophilidae), but it differs from *C. procnemis* in not having wings whitening at tip (Wheeler, 1952). The main morphological characters of *C. procnemoides* (black fore femora, tibiae and metatarsi) in the samples are shown in Figure 1.

In the present study, *C. procnemoides* was collected only from a single locality in

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Figure 1. Lateral view of *Chymomyza procnemoides* (♀) (black fore femora, tibiae and metatarsi).

Uşak province. This is the first record in both genus and species level for Turkey and we believe that it is important in terms of biodiversity of the Turkish entomofauna.

We are grateful to Dr Paul Beuk (Maastricht Natural History Museum, Maastricht, Netherlands) for the identification of the specimens.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη καταγραφή του δίπτερου *Chymomyza procnemoides* (Wheeler) (Diptera: Drosophilidae) στην εντομοπανίδα της Τουρκίας

E. Zengin

Περίληψη Αυτή είναι η πρώτη καταγραφή του δίπτερου *Chymomyza procnemoides* (Wheeler, 1952) (Diptera: Drosophilidae) στην Τουρκία. Τα δείγματα ελήφθησαν από δολωματικές παγίδες εντόμων σε οπωροφόρα δέντρα μηλιάς, αχλαδιάς και δαμασκηνιάς στην επαρχία Uşak κατά το έτος 2017.

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Investigating the *in vitro* and *in vivo* nematicidal performance of structurally related macrolides against the root-knot nematode, *Meloidogyne incognita*

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Summary Avermectins and spinosyns are structurally related natural products of microbial origin and belong to a new family of macrolides which are active against a vast array of invertebrate pests. In the present study, the effects of four members of macrolides; abamectin (ABM), emamectin benzoate (EMB), spinosad (SPI) and spinetoram (SPIT), on *Meloidogyne incognita* were investigated under *in vitro* and *in vivo* conditions. All compounds reduced egg hatching and led to high mortality of the nematode second-stage juveniles (J_2). ABM showed the maximum rate of egg hatching inhibition and J_2 mortality while SPIT recorded the minimum. All treatments reduced the number of galls, egg masses, eggs/egg mass in roots and J_2 in the soil when compared to the control. Based on the 10 folds of the 24 h-LC_{50} values of J_2 mortality *in vitro*, EMB and ABM exhibited higher percent reduction in galls (79.68 and 71.45%), egg masses (75.19 and 70.54%), eggs/ egg mass (60.49 and 40.91%) and J_2 in the soil (90.31 and 86.54%), respectively, compared to SPI and SPIT. Significant increase in tomato shoot height occurred in all biopesticides (10 folds) and SPIT (20 folds). SPI at 10 folds of the 24 h-LC_{50} values of J_2 mortality *in vitro,* significantly increased root length while ABM at 50 folds and SPIT at 20 folds decreased root length by 5.15% and 5.88%, respectively, compared to the untreated inoculated plants. In all treatments, the dry shoot and root weights increased, compared to the untreated control. Our findings suggest that these macrolides have the ability to regulate nematode population densities and may be an alternative to classical nematicides.

Additional keywords: avermectins, biopesticides, macrolides, nematicidal activity, root-knot nematodes, spinosyns

Introduction

Tomato (*Solanum lycopersicum* L.) is an important and vastly grown vegetable in Egypt and worldwide. However, its growth, yield and economic productivity are significantly reduced by pests and diseases. Plant parasitic nematodes (PPNs) are found to be the most common and destructive pests causing estimated crop losses of US $ 118 billion each year worldwide (Atkinson *et al.*, 2012). Among PPNs, *Meloidogyne* spp., root-knot nematodes, are harmful agricultural pests causing huge damage around the world (Sikora and Fernandez, 2005).

For sustainable tomato production, effective management of PPNs especially root-knot nematodes is essential. Several approaches are used to minimise PPNs in the field, including synthetic nematicides, resistant plant cultivars, botanical pesticides, antagonistic microorganisms (e.g. fungi and bacteria), beneficial fungi (Mycorrhiza), organic amendments, soil solarization and plant extracts (Collange *et al.*, 2011; D'Addabbo *et al.,* 2011; Radwan *et al.*, 2012; Saad *et al.*, 2017). Farmers rely mainly on the application of synthetic nematicides rather than on other approaches. However, lately many of these chemicals are being withdrawn from the markets due to environmental health and safety concerns (Rich *et al.,* 2004). This highlights the need for devel-

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oping environmentally safer, target-specific ways of controlling these parasites.

To date, there is an increasing interest towards the utilisation of microorganisms as biocontrol agents in sustainable agriculture as an alternative to synthetic pesticides for controlling various crop pests and diseases, as well as improving crop yield. These microorganisms produce a great variety of structurally unique bioactive secondary metabolites. For example, Actinomycetes, which are found in soil and aquatic habitats produce more than 10,000 such active compounds. Among the bacteria used as microbial antagonists, Actinobacteria, especially *Streptomyces* spp., display activity against PPN by generating nematicidal metabolites (Mishra *et al.,* 1987; Sun *et al*., 2006) and chitinolytic enzymes (Barka *et al.,* 2016).

Avermectins, a new class of 16-membered macrocyclic lactones, have four pairs of homologue compounds, *i.e.* four major components $A1_a$, $A2_a$, $B1_a$ and $B2_a$, and four minor components $A1_b$, $A2_b$, $B1_b$ and $B2_b$. Avermectins have been isolated from the crude fermentation product of *Streptomyces avermitilis* (Faske and Starr, 2007), and proved to possess a broad spectrum of pesticidal effects such as insecticidal, acaricidal, nematicidal and anthelmintic activities (Jansson and Dybas, 1998).

ABM, a blend of avermectins $B1_a$ (<80%) and $B1_b$ (>20%) with identical biological and toxicological properties (Pitterna *et al*., 2009), has nematicidal effects against root-knot and other nematode genera against several crops (Faske and Starr, 2007; Saad *et al.,* 2017). On the other hand, EMB, a second generation avermectin derivative, is being developed for control of insect pests on different vegetable crops worldwide (Jansson and Dybas, 1998). It is structurally related to ABM having higher insecticidal action than ABM. It is also effective against root-knot nematodes (Rehman *et al*., 2009).

Spinosyns are novel macrolides, natural metabolites produced under aerial fermentation conditions by the soil actinomycete *Saccharopolyspora spinosa*. This Gram-positive bacterium produces SPI, a natural pesticide which is a mixture of spinosyn A and spinosyn D (85:15), that was reported to be an effective pest control agent with low toxicity to humans and the environment (Sparks *et al.,* 1996).

SPI is toxicologically classified by the U.S. Environmental Protection Agency as a reduced risk material. SPIT is an analogue to SPI that belongs to spinosyns and it is a mixture of chemically modified spinosyns J and L. These molecules were found to have a wide spectrum of insect control potential on a variety of crops with high residual action (Huang *et al*., 2009).

Although the interest in avermectins, as one class of macrocyclic lactones (MLs) for nematicidal use, is increasing there is scarce information in the literature about the effectiveness of MLs compounds against root-knot nematodes. This encouraged us to continue investigating this group of chemicals for root-knot nematodes management. Therefore, the main goals of the present study were to assess the *in vitro* nematicidal potential of the structurally related macrolides; ABM, EMB, SPI and SPIT against *Meloidogyne incognita*. An *in vivo* pot trial was also undertaken to investigate their efficacy against the nematode on tomato under greenhouse conditions.

Materials and Methods

Macrocyclic lactones and a standard nematicide

ABM (Tervigo® 2% SC) and EMB (Proclaim® 5 % WG) were supplied by Syngenta, Egypt and SPI (Tracer® 24 % SC) and SPIT (Radient® 12 % SC) by Dow AgroSciences, Egypt and the standard nematicide oxamyl (Vydate24% SL) was supplied by E. I. du Pont de Nemours & Company Inc. was used for comparison.

Root-knot nematode inocula

A single egg mass was excised from the roots of an infested eggplant (*Solanum melongena* cv. Black Beauty) and a pure culture of the root knot nematode isolate was propagated on the roots of tomato (*S. lycopersicum*

cv. Golden Stone) under greenhouse conditions. The population was eventually identified as *Meloidogyne incognita*, according to Taylor and Nelscher (1974) using perineal patterns. During the course of this study, eggs were being extracted from infected roots with sodium hypochlorite (NaOCl) according to Hussey and Barker (1973) and second stage juveniles (J_2) obtained using the Baermann plate technique (Ayoub, 1980).

In vitro assays

The assessment of the effect of ABM, EMB, SPI and SPIT on hatching and mortality of *M. incognita* J_2 was carried out *in vitro*. Preliminary experiments were conducted to establish the effective range of concentrations of the chemicals.

Hatching assays

The concentrations of each chemical tested during this study were as follows: for ABM and EMB, 25, 50, 100, 200 and 400 mg/l; for SPI, 250, 500, 1000, 2000 and 3000 mg/l and for SPIT, 250, 500, 1000, 1500 and 2000 mg/l. Vials (each one ca. 15 ml) containing distilled water served as control. Each treatment was replicated four times and each replicate involved approximately 1200 eggs. The numbers of J_2, hatched from eggs, were recorded at 3 and 7 days after application.

Mortality assays

The concentrations of each chemical tested during this study were as follows: for ABM, 12.5, 25, 50, 75 and 100 mg/l; for EMB, 25, 50, 75, 100 and 200 mg/l; for SPI, 250, 500, 1000, 1500 and 3000 mg/l and for SPIT, 250, 500, 1000, 1500 and 2000 mg/l. Each treatment was replicated four times including distilled water as a control and each replicate involved 200 J_2. The numbers of both dead and alive J_2 were recorded after 24 and 48 h exposure and the mortality percentages was estimated.

Pot assay

The nematicidal performance of ABM, EMB, SPI and SPIT was tested on tomato plants infested with *M. incognita*. Pots with capacity of one kg soil were filled with autoclaved loamy sand soil. ABM and EMB were applied as a soil drench at the rate of 10 and 50 folds of their LC_{50}'s values based on J_2 mortality test after 24 h exposure, while SPI and SPIT were applied at the rate of 10 and 20 folds of their LC_{50}'s values after 24 h exposure. Oxamyl was used as a standard nematicide.

One one-month old tomato seedling cv. HERMIS was transplanted in each pot, and three days later inoculated with 5000 eggs. Untreated uninoculated and untreated inoculated plants served as controls. All treatments were replicated five times and arranged in a complete randomized design on a bench in a greenhouse (28 ± 2°C, 65 ± 2 RH and 12: 12 L:D photoperiod). During the course of the experiment, irrigation and fertilization were applied when appropriate. Fifty days after the inoculation, the plants were removed and washed free of soil. Shoot height and dry weight, root length and dry weight were measured and number of galls/root system, egg-masses/root system, eggs/egg-mass and J_2/250g soil were estimated. J_2s were extracted as previously mentioned and Phloxine B was used to stain the roots to facilitate egg mass counting (Holbrook *et al*., 1983).

Statistical analysis

The statistical analysis of data was carried out using a computer Costat program (2005) version 6.303. Statistically significant differences between the means were compared using analysis of variance (ANOVA) with the least significant differences (LSD) and *P*-values at 0.05 probability. Hatching and J_2 mortality percentages were estimated using the Abbott formula (1925), and Probit analysis was used to calculate LC_{50} for each compound according to Finney (1971).

Results

Impact of test compounds on egg hatching and J_2 mortality of *M. incognita* under laboratory conditions

The egg hatching inhibition rate (%) un-

der laboratory conditions, due to exposure to the tested bioproducts after two time intervals is illustrated in Fig. (1). Hatching was inversely proportional to the concentration of the bioproducts. After 3 and 7 days exposure, the most effective compounds causing hatching reduction were ABM (96.32 and 85.41%, respectively) and EMB (88.55% and 71.23%, respectively) at 400 mg/l. At 2000 mg/l, hatching inhibition was 73.83% and 69.40% for SPI and 77.72% and 73.35% for SPIT (Fig. 1). LC_{50} values on hatching inhibition after 3 and 7 days exposure were respectively, for ABM 24.61 mg/l and 46.89 mg/l, for EMB 47.97 mg/l and 83.09 mg/l, for SPI 629.53 mg/l and 781.52 mg/l and for SPIT, 487.46 mg/l and 635.66 mg/l (Table 1).

J_2 mortality increased by increasing compound concentration and exposure time, whereas no mortality occurred in the controls. After 24 and 48 h exposure, J_2 mortality for ABM at 100 mg/l was 73.01% and 86.00%, respectively, and for EMB 51.43% and 63.08%, respectively. SPI at 1500 mg/l caused a 45.22% and 50.66% mortality, while SPIT 32.86% and 42.03%, respectively. This indicates that there is a marked increase in J_2 mortality caused by ABM over EMB and by SPI over SPIT (Fig. 2). Probit analysis of these results indicates that, after 24 h exposure, ABM was the most toxic compound against J_2 (LC_{50} = 36.64 mg/ml) followed by EMB, SPI and SPIT. LC_{50} values after 48 h exposure were 22.89, 79.03, 1611.27 and 2355.52 mg/l for ABM, EMB, SPI and SPIT, respectively. In general, these compounds could be arranged according to their effectiveness on J_2 mortality as follow: ABM > EMB > SPI > SPIT (Table 1).

Effect of test compounds against *M. incognita* at pot assay

All treatments showed differential nematicidal properties when compared to the untreated inoculated control. Gall formation was significantly suppressed by EMB, ABM, SPI and SPIT with reductions of 71.65, 69.46, 64.54 and 64.01%, respectively. However, no significant differences were observed between ABM and EMB and between SPI and SPIT (Table 2). Except for EMB, no significant differences were observed between the lower and the higher rates of ABM, SPI and SPIT. The same trend was exhibited with respect to egg masses/root system. EMB was the most effective followed by ABM, SPI and SPIT, reducing egg masses by 76.28, 74.57, 56.20 and 51.24%, respectively. No significant differences were detected between the lower and higher rates of all treatments. With respect to the number of eggs/egg mass, EMB, SPI, ABM and SPIT recorded reductions of 61.71, 54.08, 52.34 and 45.61%, respectively. The application of EMB, ABM, SPI and SPIT suppressed population density in soil by 91.82, 89.26, 74.33 and 72.64%, respectively, compared to the control. No significant differences were observed between the lower and the higher rates of all applied treatments (Table 2).

The effect of ABM, EMB, SPI and SPIT as a soil drench on the shoots and roots of the tomato seedlings is shown in Table 3. Shoot height increased in all the treated plants by 23.35% to 48.24%. The maximum increase was observed in plants treated with SPIT, followed by SPI, EMB and ABM. No significant differences were observed between the lower and the higher rates of all treatments. Noticeable increases were also recorded in the mean root length of plants treated with SPI, SPIT and EMB, i.e. 19.85%, 8.82% and 4.41%, respectively, whereas ABM reduced root length by 4.78%. Noticeably, the higher rate of SPIT exhibited a root length reduction by 5.88% (Table 3).

Regarding dry shoot weight, data indicate an increase as compared to the control; the highest dry weight was observed with SPI (43.46%), followed by SPIT (34.11%), ABM (16.93%) and EMB (16.54%). ABM at the lower rate (10 folds) decreased dry shoot weight by 8.97%. Plants treated with ABM showed significant differences between the lower and the higher rates, whereas no significant differences were found between the lower and the higher rates of EMB, SPI and SPIT (Table 4). All treatments recorded an increase in dry root weight over the untreated inoculated control. Such increase was min-

imum (14.26%) in plants treated with ABM, while treatment with SPI induced the maximum increase (74.26%) over the control (Table 4).

Discussion

The present investigation revealed that the tested MLs compounds possess nematicidal properties against *M. incognita* under laboratory and greenhouse conditions with the following descending order ABM >EMB >SPI> SPIT. The findings of the present *in vitro* studies are in conformity with previous studies in which ABM nematode toxicity was higher than that of EMB. ABM was more effective than EMB on hatching inhibition and juveniles mortality of *M. incognita* in laboratory tests (Ullah *et al*., 2015). ABM has been found more toxic than EMB with respect to the number of galls and egg masses in roots, with 61.77 and 78.82%, and 43.75 and 56.41% reductions, respectively (Shahid *et al*., 2009). d'Errico *et al.* (2017) reported that Tervigo® (ABM 2% SC) and two other formulations, CHA 2061-02 EW and CHA 2080 SC, showed a nematostatic activity against *M. incognita* J_2 *in vitro*, where after exposure to these products, J_2 were immobilized and subsequently resumed mobility over time following a recovery test. AVM B_1 when used at 10 and 100 mg/l completely inhibited egg hatchability of *Meloidogyne arenaria* Chitwood *in vitro* (Cayrol *et al.,* 1993). Avicta® containing ABM reduced hatching and increased *M. javanica* J_2 mortality *in vitro*. In addition to the nematostatic effect, Avicta® possessed a nematicidal effect (Almeida *et al.,* 2017). However, while studying the toxicity of EMB and ABM to *M. incognita* juveniles in the laboratory, Ding *et al.* (2009) reported that the toxicity of EMB was found higher than that of ABM, their LC_{50} being 0.1645 and 0.4532 mg/l, respectively. Also, EMB was highly toxic to *M. incognita* juveniles with LC_{50} and LC_{90} values of 3.59 and 18.20 mg/L after 48 h of exposure, respectively (Cheng *et al.,* 2015).

Indeed, avermectins have already been proven nematicidal and effective in reducing nematode populations both in soil and the roots of infested plants. Regardless the method of application, our findings confirmed published reports in which ABM was effective against root-knot nematodes on cotton (Faske and Starr, 2007), tomato (Qiao *et al,* 2012; Ullah *et al.,* 2015; Saad *et al.,* 2017) and cucumber (Huang *et al.,* 2014). Nursery bed soil drenching with EMB 1.9 % WP at 285.0 g a.i./ha before or after sowing, induced high reduction of the J_2 population in the soil as well as of the number of females per g root (Das *et al.,* 2014).

SPIT is often more potent, faster-acting, and longer-lasting than SPI as an insecticide (Sparks *et al.,* 2008; Dripps *et al*., 2011). In the present study, spinosyn compounds displayed satisfactory results regarding the nematicidal activity against *M. incognita*, both under *in vitro* and *in vivo* conditions. However, their nematicidal efficacy was lower than that of the avermectin compounds. To our knowledge, the potency of spinosyn against PPNs has not been reported yet, except for the effect of SPI as a nematicide recorded by Khalil (2013) where Tracer® 24% SC at 0.5 and 0.1% reduced *M. incognita* populations by 70.90 and 62.51%, respectively.

The increase in plant growth parameters, such as shoot height, root length and dry weight of either shoots and roots suggests that the treatments tested during this study have a good potential nematicidal effect on the root knot nematode *M. incognita*, which can result in effective plant protection. The obtained results are consistent with the earlier report by Ding *et al.* (2009) that proved the effectiveness of EMB in improving plant growth of tomato. Such improvement in plant growth is possibly due to the reduction in PPN populations. Our findings are also in agreement with the data of Khalil (2012) and Saad *et al.* (2017), who found that ABM when applied against *M. incognita* infesting tomato plants, increased all plant growth parameters. Moreover, ABM enhanced cucumber plant vigor and fruit yield (Huang *et al.*, 2014). However, Khalil (2013) found that SPI at 0.1% reduced the fresh weight of roots by 20.69% when ap-

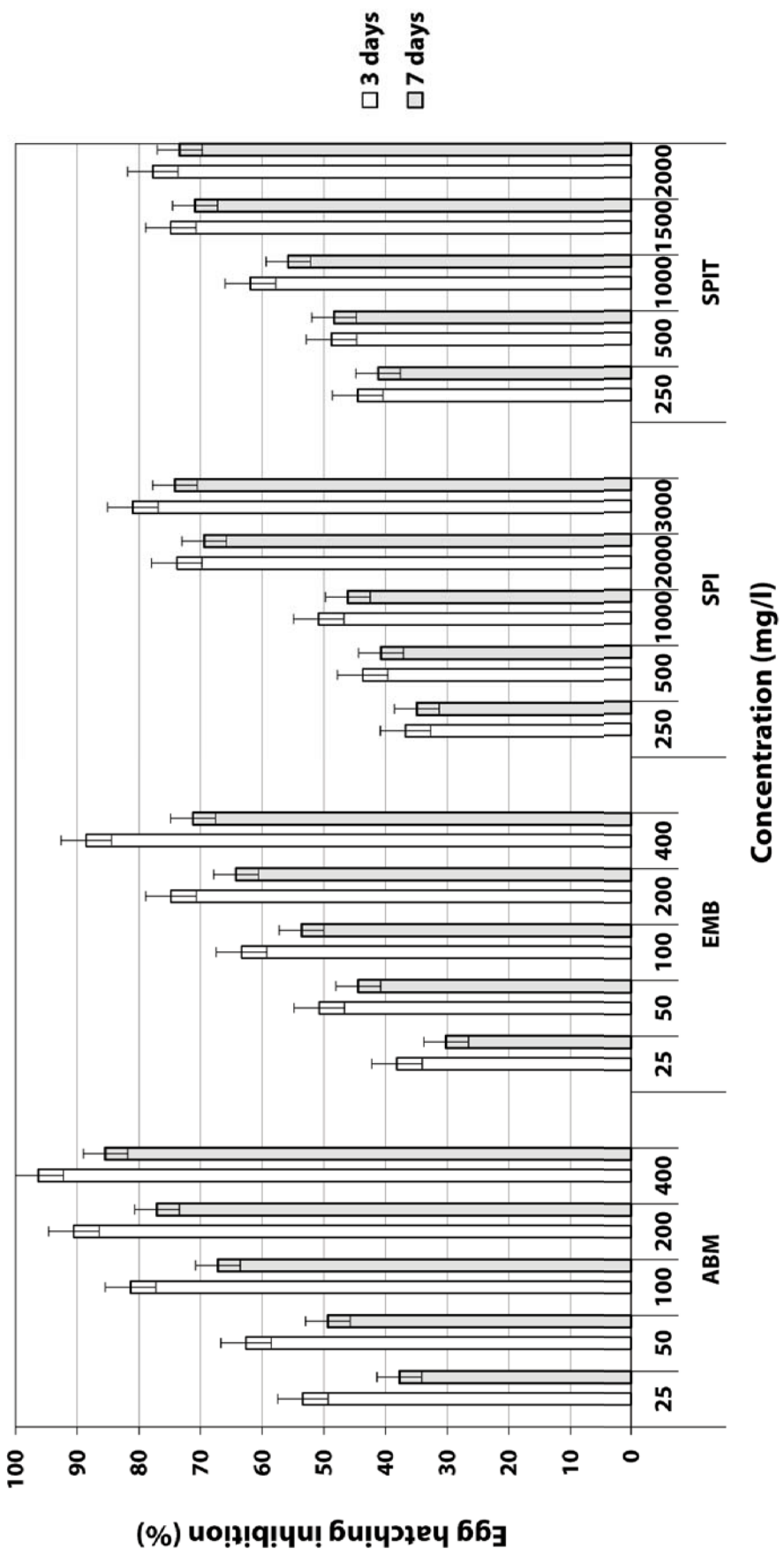

Figure 1. Egg hatching inhibition (mean of 4 replications) of *Meloidogyne incognita* exposed to different concentrations of abamectin (ABM), emamectin (EMB), spinosad (SPI) and spinetoram (SPIT) after 3 and 7 days of exposure under laboratory conditions.

Table 1. Effect of abamectin (ABM), emamectin (EMB), spinosad (SPI) and spinetoram (SPIT) on egg hatching inhibition and juvenile mortality of the root-knot nematode *Meloidogyne incognita* at two time intervals.

Treatments	Exposure times							
	Egg hatching inhibition (days)				J_2 Juvenile mortality (hrs)			
	3 days				24 hrs			
	LC_{50} (mg/l)	Fiducial Limits (Lower – Upper)	Slop ± variance	Regression equation	LC_{50} (mg/l)	Fiducial Limits (Lower – Upper)	Slop ± variance	Regression equation
ABM	24.61	(21.18 - 28.57)	1.43 ± 0.006	Y = -1.99 + 143 X	36.64	(31.50 - 42.60)	1.28 ± 0.01	Y = -2.00 + 1.28 X
EMB	47.97	(41.31- 55.67)	1.19 ± 0.006	Y = - 2.00 + 1.19 X	111.62	(94.98 - 131.28)	1.41 ± 0.02	Y = -2.88 + 1.41X
SPI	487.46	(425.95 - 557.70)	1.23 ± 0.008	Y = -3.40 + 1.22 X	2558.07	(1966.31 - 3331.8)	1.12 ± 0.01	Y = -3.82 + 1.12 X
SPIT	629.53	(563.36 - 703.37)	1.14 ± 0.004	Y = -3.19 + 1.14 X	3077.10	(2099.84 - 4518.96)	1.02 ± 0.01	Y = -3.57 + 1.02 X
	7 days				48 hrs			
ABM	46.89	(41.77 - 52.62)	1.17 ± 0.003	Y = -1.95 + 1.17 X	22.89	(19.21 - 27.22)	1.36 ± 0.01	Y = -1.85 + 1.36 X
EMB	83.09	(72.16 - 95.65)	0.88 ± 0.004	Y = - 1.70 + 0.88 X	79.03	(69.13 - 90.35)	1.5 ± 0.02	Y = -2.84 + 1.50 X
SPI	635.66	(566.50 - 713.17)	1.17 ± 0.007	Y = -2.91 + 1.01 X	1611.27	(1296.81 -2003.44)	1.04 ± 0.01	Y = -3.33 + 1.04 X
SPIT	781.52	(700.45 - 871.89)	1.01 ± 0.003	Y = -2.88 + 1.08 X	2355.52	(1738.75 - 3195.16)	1.09 ± 0.01	Y = -3.69 + 1.09 X

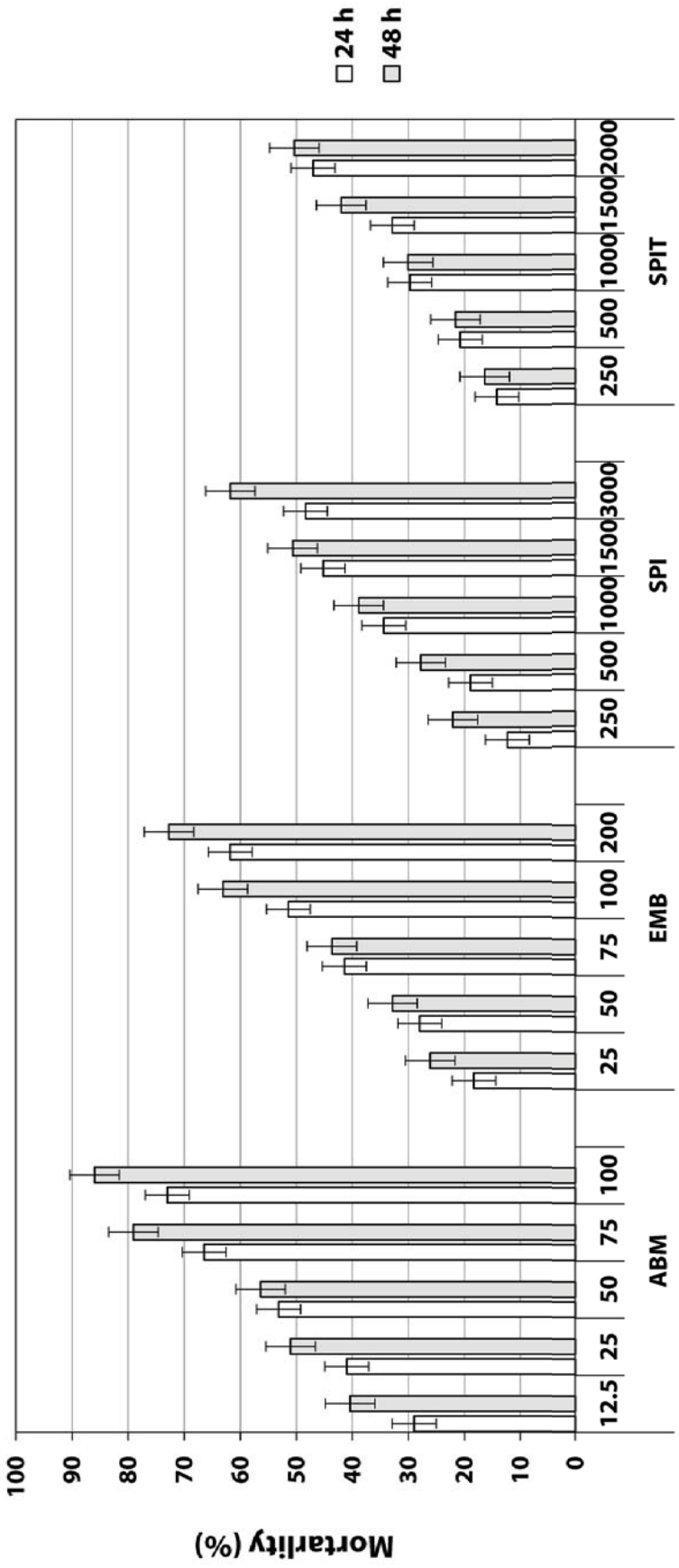

Figure 2. Mortality percentage (mean of 4 replications) of *Meloidogyne incognita* J_2 exposed to different concentrations of abamectin (ABM), emamectin (EMB), spinosad (SPI) and spinetoram (SPIT) at two time intervals under laboratory conditions.

Table 2. Effect of abamectin (ABM), emamectin (EMB), spinosad (SPI) and spinetoram (SPIT) on galls, egg masses, eggs/mass and juveniles of the root-knot nematode *Meloidogyne incognita,* on tomato.

Treatments 24 h-LC_{50} (mg/l) of J_2	Rate/kg soil	Galls			Egg masses			Eggs/ Mass			Juveniles / 250g soil		
		Mean ± SE	[b]R (%)	[c]MR (%)	Mean± SE	R (%)	MR (%)	Mean ± SE	R (%)	MR (%)	Mean ± SE	R (%)	MR (%)
Untreated control	-	150.60 ± 6.75 [a]	-	-	129.00 ± 7.24[a]	-	-	676.77± 17.99[a]	-	-	897.80 ± 26.31[a]	-	-
ABM (36.64 mg/l)	[a]10 fold	43.00 ±5.08[cd]	71.45	69.46	38.00 ±4.16[c]	70.54	74.57	399.90± 15.45[b]	40.91	52.34	120.80± 9.07[d]	86.54	89.26
	50 fold	49.00 ± 4.70[bc]	67.46		27.60± 3.31[c]	78.60		245.23 ± 11.69[g]	63.76		72.00± 9.56[de]	91.98	
EMB (111.62 mg/l)	10 fold	30.60± 3.43[de]	79.68	71.65	32.00 ± 3.01[c]	75.19	76.28	267.40 ± 9.66[efg]	60.49	61.71	87.00 ±9.05[de]	90.31	91.81
	50 fold	54.80 ± 5.08[bc]	63.61		29.20±3.50[c]	77.36		250.93 ± 8.45[fg]	62.92		60.00 ±6.27[e]	93.32	
SPI (2558.07 mg/l)	10 fold	59.20 ± 5.62 [bc]	60.69	64.54	57.60 ±5.45[b]	55.35	56.20	327.20 ± 11.38[cd]	51.65	54.07	244.60± 10.65[b]	72.76	74.33
	20 fold	47.60 ± 4.02[bcd]	68.39		55.40 ±4.83[b]	57.05		294.42 ± 9.48[def]	56.50		216.40± 10.07[bc]	75.90	
SPIT (3077.10 mg/l)	10 fold	61.80 ± 3.79[b]	58.96	64.01	70.00 ±5.30[b]	45.74	51.24	437.20 ± 11.31[b]	35.40	45.61	256.20 ±10.96[b]	71.46	72.64
	(20 fold	46.60 ± 3.58[bcd]	69.06		55.80 ±5.81[b]	56.74		298.97 ± 12.11[de]	55.82		235.00 ± 8.20[bc]	73.82	
Oxamyl	0.05 ml	23.60 ± 2.87[e]	84.33	84.33	25.40± 3.60[c]	80.31	80.31	347.63 ± 14.67[c]	48.63	48.63	180.60 ±15.00[c]	79.88	79.88
$LSD_{0.05}$		17.22	-	-	15.35	-	-	44.64	-	-	58.83	-	-

Values are means of five replicates ± SE. Values in each column followed by the same letter(s) are not significantly different according to LSD (p = 0.05)
[a]10, 20 and 50 fold were calculated based on the 24 h-LC_{50}value (mg/l) of J_2 juvenile mortality *in vitro*, [b]R(%): Reduction percentage, [c]MR(%): The average reduction percentage.

Table 3. Impact of abamectin (ABM), emamectin (EMB), spinosad (SPI) and spinetoram (SPIT) on shoot height and root length of tomato plants infected with the root-knot nematode *Meloidogyne incognita*.

Treatments 24 h-LC_{50} (mg/l) of J_2	Rate/kg soil	Shoot height (cm)			Root length (cm)		
		Mean ± SE	Increase (%)	[b]MI (%)	Mean ± SE	Increase (%)	MI (%)
Untreated inoculated plants	-	22.70± 0.95^{d}	-	-	13.60± 0.82^{de}	-	-
Untreated uninoculated plants	-	30.10±1.30^{abc}	32.60	32.60	23.20±1.31^{a}	70.59	70.59
ABM (36.64 mg/l)	[a]10 fold	31.50± 1.23^{abc}	38.77	23.35	13.00± 0.95^{e}	-4.41	-4.78
	50 fold	24.50± 1.20^{cd}	7.93		12.90±1.18^{e}	-5.15	
EMB (111.62 mg/l)	10 fold	31.50±1.31^{abc}	38.77	29.74	13.60± 0.99^{de}	0.00	4.41
	50 fold	27.40± 1.26^{bcd}	20.70		14.80±1.00^{cde}	8.82	
SPI (2558.07 mg/l)	10 fold	32.70± 1.29^{ab}	44.05	37.22	17.70±0.95^{bc}	30.15	19.85
	20 fold	29.60± 1.08^{abcd}	30.40		14.90± 0.70^{cde}	9.56	
SPIT (3077.10 mg/l)	10 fold	36.30± 1.28^{a}	59.91	48.24	16.80± 0.73^{bcd}	23.53	8.82
	20 fold	31.00±0.98^{abc}	36.56		12.80± 0.54^{e}	-5.88	
Oxamyl	0.05 ml	29.70±1.29^{abcd}	30.84	30.84	20.00± 1.32^{ab}	47.06	47.06
$LSD_{0.05}$		7.00	-	-	3.45	-	-

Values are means of five replicates ± SE. Values in each column followed by the same letter(s) are not significantly different according to LSD (p = 0.05)
[a]10, 20 and 50 fold were calculated based on the 24 h-LC_{50} value (mg/l) of J_2 juvenile mortality *in vitro*, [b]MI(%)= average increase percentage.

Table 4. Dry weight of shoot and root system of tomato plants infected with the root-knot nematode *Meloidogyne incognita*, after treatment with abamectin (ABM), emamectin (EMB), spinosad (SPI) and spinetoram (SPIT).

Treatments 24 h-LC_{50} (mg/l) of J_2	Rate/kg soil	Shoot dry weight (g)			Root dry weight (g)		
		Mean ± SE	Increase (%)	[b]MI (%)	Mean ± SE	Increase (%)	MI (%)
Untreated inoculated plants	-	0.78± 0.07^{cd}	-	-	0.47±0.03^{d}	-	-
Untreated uninoculated plants	-	1.02±0.04^{abc}	30.77	30.77	0.57± 0.08^{bcd}	20.43	20.43
ABM (36.64 mg/l)	[a]10 fold	0.71±0.05^{d}	-8.97	16.92	0.48± 0.04^{d}	1.28	14.26
	50 fold	1.11±0.09^{ab}	42.82		0.60± 0.05^{bcd}	27.23	
EMB (111.62 mg/l)	10 fold	0.84±0.07^{cd}	7.18	16.54	0.74± 0.10^{abc}	58.30	66.38
	50 fold	0.98±0.06^{abc}	25.90		0.82±0.08^{a}	74.47	
SPI (2558.07 mg/l)	10 fold	1.14±0.15^{ab}	45.64	43.46	0.72±0.08^{abc}	54.04	74.26
	20 fold	1.10±0.05^{ab}	41.28		0.91±0.05^{a}	94.47	
SPIT (3077.10 mg/l)	10 fold	1.17± 0.12^{ab}	50.26	34.10	0.78±0.03^{ab}	65.11	40.21
	20 fold	0.92± 0.05^{bcd}	17.95		0.54±0.06^{cd}	15.32	
Oxamyl	0.05 ml	1.21±0.07^{a}	54.62	54.62	0.76±0.08^{ab}	61.70	61.70
$LSD_{0.05}$		0.26		-	0.21	-	-

Values are means of five replicates ± SE. Values in each column followed by the same letter(s) are not significantly different according to LSD (p = 0.05).
[a]10, 20 and 50 fold were calculated based on the 24 h-LC_{50} value (mg/l) of J_2 juvenile mortality *in vitro*, [b]MI(%)= average increase percentage.

plied against *M. incognita* on tomatoes. The decrease in some plant growth parameters in the present study may be attributed to phytotoxicity.

Overall, the tested avermectins and spinosins can be considered as interesting alternative tools for the management of the root-knot nematode, *M. incognita*, being compounds with a good nematicidal potential, which have different mode of action to the available nematicides (Salgado, 1998; Bloomquist, 2003; Watson *et al*., 2010).

Conclusions

The current study provides evidence that the structurally related macrocyclic lactone compounds ABM, EMB, SPI and SPIT have a good potential to control the population of the root-knot nematode, *M. incognita*, by reducing hatching and increasing J_2 mortality *in vitro*. Also, soil drenching with these compounds significantly reduced the reproduction of *M. incognita* and consequently enhanced tomato growth characters. ABM and EMB as members of avermectins had greater efficacy on the *M. incognita* than SPI and SPIT as members of spinosyns. In general, the tested compounds are promising alternatives (bionematicides) to the classical nematicides for the control of root-knot nematodes in tomato production. Nevertheless, further research is required to assess the nematicidal properties of these compounds under field conditions. Furthermore, future research can extend to designing new controlled release formulations based on a nano-delivery system, which would enhance their efficacy and expand their use in the area of PPN management.

Declaration of Conflicting Interests

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Διερεύνηση της *in vitro* και *in vivo* νηματωδοκτόνου δράσης δομικά συγγενών δραστικών ουσιών της Ομάδας των μακρολιδίων έναντι του κομβονηματώδη *Meloidogyne incognita*

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Περίληψη Οι αβερμεκτίνες και οι σπινοσίνες είναι δομικά συγγενή φυσικά προϊόντα μικροβιακής προέλευσης και ανήκουν σε μια νέα οικογένεια μακρολιδίων με δράση έναντι ενός μεγάλου εύρους ασπόνδυλων φυτοπαρασίτων. Στην παρούσα μελέτη διερευνήθηκε, σε συνθήκες *in vitro* και *in vivo*, η επίδραση τεσσάρων τέτοιων δραστικών, της αβαμεκτίνης (ABM), της βενζοϊκής εμαμεκτίνης (EMB), του spinosad (SPI) και του spinetoram (SPIT), στον κομβονηματώδη *Meloidogyne incognita*. Όλες οι ενώσεις μείωσαν την εκκόλαψη ωών και οδήγησαν σε υψηλή θνησιμότητα των προνυμφών 2ου σταδίου (J_2) του νηματώδη. Η αβαμεκτίνη κατέδειξε τα μεγαλύτερα ποσοστά αναστολής εκκόλαψης ωών και θνησιμότητας προνυμφών J_2, ενώ το SPIT κατέγραψε το μικρότερο ποσοστό. Όλες οι επεμβάσεις μείωσαν τον αριθμό όγκων (κόμβων), ωών, ωών/ ωόσακους στις ρίζες και προνυμφών J_2 στο έδαφος, σε σύγκριση με το μάρτυρα. Η βενζοϊκή εμαμεκτίνη και η αβαμεκτίνη, σε συγκέντρωση δεκαπλάσια της τιμής LC_{50} για τη θνησιμότητα των προνυμφών J_2 *in vitro* στις 24 ώρες, εμφάνισαν υψηλότερη ποσοστιαία μείωση κόμβων (79,68 και 71,45%), ωόσακων (75,19 και 70,54%), ωών/ ωόσακους (40,91%) και προνυμφών J_2 στο έδαφος (90,31 και 86,54%), αντίστοιχα, σε σύγκριση με το SPI και το SPIT. Επίσης, παρατηρήθηκε σημαντική αύξηση στο ύψος των βλαστών της τομάτας σε όλες τις δραστικές ουσίες (Χ 10 φορές) και στο SPIT (Χ 20 φορές). Το μήκος της ρίζας αυξήθηκε σημαντικά από το SPI σε 10 πλάσια συγκέντρωση της τιμής LC_{50} για τη θνησιμότητα των προνυμφών J_2 *in vitro* στις 24 ώρες ενώ μειώθηκε από την αβαμεκτίνη σε 50 πλάσια συγκέντρωση και το SPIT σε 20 πλάσια συγκέντρωση, κατά 5,15% και 5,88% αντίστοιχα, σε σχέση με τα φυτά του μάρτυρα (χωρίς επέμβαση). Το ξηρό βάρος βλαστών και ριζών αυξήθηκε σε όλες τις επεμβάσεις σε σύγκριση με το μάρτυρα. Τα ευρήματα υποδεικνύουν ότι οι ενώσεις αυτές έχουν την ικανότητα να ρυθμίζουν την πληθυσμιακή πυκνότητα των νηματωδών και μπορεί να αποτελέσουν μια εναλλακτική λύση έναντι των κλασικών νηματοδοκτόνων.

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Contents

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