Lethal and sublethal effects of several plant compounds compared to spiromesifen against *Tetranychus turkestan*

F. Sohrabi^{1*} and M. Ziaee²

Summary *Tetranychus turkestan* Ugarov and Nikolski is one of the main agricultural pests of south-western Iran and some other tropical regions. In the present study, fumigant activity of three essential oils extracted from *Rosmarinus officinalis* L., *Mentha longifolia* L. and *Eucalyptus globulus* Labill., and contact toxicity of two biopesticides (Tondexir and Palizin) on *T. turkestan* mite females were investigated and compared with that of spiromesifen, a chemical acaricide. Also, sublethal effects of the tested compounds at 50% lethal concentration (LC₅₀) were estimated on the biological parameters of *T. turkestan*. The LC₅₀ values for *E. globulus*, *R. officinalis* and *M. longifolia* essential oils were 12.50, 11.52 and 4.00 µl/l air and for spiromesifen, Tondexir and Palizin were 10.98, 327.34 and 858.13 ppm, respectively. All tested compounds significantly reduced adult female longevity, equally to the chemical acaricide spiromesifen. Fecundity also decreased in all treatments and this reduction was even higher for plant essential oils than the other compounds. Palizin, *E. globulus* and *M. longifolia* significantly reduced the hatchability of *T. turkestan* eggs similarly to spiromesifen. According to the results, the tested plant compounds are effective against *T. turkestan* and may be applied as suitable alternatives to synthetic pesticides against this crop pest.

Additional keywords: biological parameters, biopesticide, Palizin, spider mite, Tondexir

Introduction

Tetranychus turkestan Ugarov and Nikolski, strawberry spider mite, (Acari: Tetranychidae) is a severe pest in some parts of tropical regions and southwestern Iran (Zhang, 2003; Karami-Jamouri and Shishehbor, 2012). It is a polyphagous pest and has been reported from 270 host plants worldwide (Jeppson *et al.*, 1975; Migeon and Dorkeld, 2006-2013). The mite feeds in a piercing-sucking manner on the underside of leaves, covers leaves by web, thus destroying plant cells and tissue. The feeding causes yellow chlorotic spots on leaves and decreases photosynthetic rate of plants (Martinez-Ferrer *et al.*, 2006; Mohammadi *et al.*, 2015).

In Iran, synthetic acaricides are mainly

applied against spider mites in various crops (Nikpay *et al.*, 2016; Morteza *et al.*, 2017; Ziaee *et al.*, 2017). However, high propagation potential, short generation time, and arrhenotokous parthenogenesis, combined with repeated acaricide use, led to the *build-up* of resistance among mite populations. Finding alternative control methods that are sustainable and environmentally friendly is necessary (Lee *et al.*, 2003; Van Leeuwen *et al.*, 2005; Pree *et al.*, 2005; Osakabe *et al.*, 2009).

Plant essential oils can be considered as an alternative for controlling spider mites, due to their compatibility with biological control agents and shorter residual effect, while they are safer to humans (Chiasson *et al.*, 2004; Hincapié *et al.*, 2008; Hussein *et al.*, 2013). As a result, the application of botanical pesticides and their natural compounds has been considered for pest control in recent years (Isman, 2000). *Rosmarinus officinalis* L., *Mentha longifolia* L. (both Lamiaceae) and *Eucalyptus globulus* Labill. (Myrtaceae) are considered as three of the most valuable aromatic and medicinal plants. The lethal and sublethal impact of essential oils extracted

¹ Department of Plant Protection, Faculty of Agriculture, Persian Gulf University, Bushehr, Iran, P.O. Box 75169-13798.

² Department of Plant Protection, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

* Corresponding author: f.sohrabi1361@gmail.com
fsohrabi@pgu.ac.ir

from these plants have been demonstrated on tetranychid mites in the past. Miresmaili *et al.* (2006) and Choi *et al.* (2004) pointed out that *R. officinalis* essential oils was effective against the phytophagous mite *Tetranychus urticae* Koch. Motazedian *et al.* (2012) stated that the essential oil of *M. longifolia* possesses a repellent effect against *T. urticae*. The results of repellent, antifeedent and toxic effects of *E. globulus* essential oil on *T. urticae* showed that *E. globulus* essential oil can be considered as a natural agent against *T. urticae* (Hussein *et al.*, 2013).

Botanical pesticides have also been reported as a source of bioacaricides for the control of tetranychid mites (Azaizeh *et al.*, 2007; Nikpay *et al.*, 2016). Tondexir contains pepper extract (TON) and Palizin is coconut extract (PAL). They are two botanical pesticides recently used in Iran against various pests, including spider mites (Kabiri *et al.*, 2013; Honarmand *et al.*, 2016; Mirfakhraie and Mohamadian, 2017; Sohrabi *et al.*, 2019).

Evaluation of lethal and sublethal effects of any pesticide on spider mites is essential for assessing the toxicity of the pesticide and probability of resistance development in the mite population (Stark and Banks, 2003; Mohammadi *et al.*, 2016). Therefore, in this study, the lethal and sublethal effects of several plant compounds including *R. officinalis*, *M. longifolia*, and *E. globulus* essential oils and two biopesticides (Tondexir and Palizin) were evaluated against *T. turkestanii* females and compared with that of Spiromesifen, a commercial chemical acaricide.

Materials and Methods

Mite culture

Tetranychus turkestanii population used for the bioassays was reared in the laboratory on cowpea, *Vigna unguiculata* (L.) Walp. at 27±1°C, 65±5% R.H. and a 16:8 L:D photoperiod.

Test compounds

Rosmarinus officinalis, *M. longifolia*, and *E. globulus* essential oils of 100% puri-

ty were tested which were provided by the Essential Herbal Pharmaceutical Company (Golestan, Iran). The bioacaricides used were Palizin SL 65% (PAL) and Tondexir EC 85% (TON), provided by Kimia Sabzavar Co., Iran. Spiromesifen, as commercial formulation Oberon® (SC 24%; Bayer CropScience), was purchased from Giah Bazr Alvand Company, Iran.

Bioassay tests

Fumigant toxicity of R. officinalis, M. longifolia, and E. globulus essential oils on adult females of T. turkestanii.

The fumigant toxicity of the essential oils was assessed in plastic Petri dishes (6 cm diameter, which offers 75 ml air space). Leaf discs of bean plants (3 cm in diameter) were placed with their dorsal side inside the Petri dishes on wet cotton. Ten adult females of mite were separately placed on the leaf plant discs using a soft paintbrush. Filter paper disks (Whatman No. 1, 2 cm in diameter) were treated with concentrations of the essential oils and placed in the Petri dishes. Five concentrations containing 1.33, 6.67, 13.33, 33.33, 66.67 µl/l air of *R. officinalis* and *E. globulus*, and 0.13, 1.33, 6.67, 13.33, 26.67 µl/l air of *M. longifolia* were used for the experiments with three replications, based on preliminary experiments and acetone was applied as a solvent. The caps of the Petri dishes were covered with parafilm to hinder the release of essential oils. In the control, filter papers were treated with acetone alone. The mortality was estimated using a stereomicroscope after 24 h of exposure. Mites were considered as dead, when no reaction was observed after fine paintbrush stimulation. The bioassays were conducted at 27±1°C, 65±5% R.H. and a 16:8 L:D photoperiod.

Contact toxicity of Tondexir, Palizin, and spiromesifen acaricides against adult females of T. turkestanii

Based on preliminary experiments, five concentrations containing 200, 320, 500, 900, 1500 ppm for TON and 100, 250, 500, 1000, 2500 ppm for PAL, and 10, 100, 250,

500, 1000 ppm in case of spiromesifen were used against the adult females of mites. Leaf discs of bean (3 cm diameter) were immersed in acaricide concentrations for 10 seconds and distilled water served as the control treatment. They were subsequently dried at room temperature for 30 min, then transferred in Petri dishes (6 cm in diameter) on wet cotton. Three replications were used for each concentration. Then, ten adult females were introduced into each Petri dish, ventilated through a hole of 1-cm diameter that was covered with organza net. Experiments were conducted at $27\pm1^{\circ}\text{C}$, $65\pm5\%$ R.H. and a photoperiod of 16:8 L:D in a growth chamber. Mortality was determined under a stereomicroscope after 24 h of exposure.

Sublethal effects

Sublethal effects of the six tested compounds at 50% lethal concentration (LC_{50}) on biological parameters of *T. turkestan*, including adult longevity, fecundity and hatchability were studied. About 100 adult females (<24 h old) were exposed to LC_{50} of each essential oil (12.50, 11.52 and $4.00\ \mu\text{l/l}$ for *E. globulus*, *R. officinalis*, and *M. longifolia*, respectively) and acaricide (10.98, 327.34 and 858.13 ppm for spiromesifen, TON and PAL, respectively) using the bioassays described above. Untreated mites were used as control. After 24 h, 20 alive *T. turkestan* females were randomly selected for each treatment and transferred individually to new untreated leaves inside Petri dishes on water-soaked cotton. Leaf discs were provided daily for each female mite (Li *et al.*, 2017). Then the adult longevity, oviposition and egg hatch were estimated. To study each compound's sublethal effects on the hatching rate, 30 eggs in six replicates were randomly selected and incubated under the conditions described above for hatching. Experiments were conducted at $27\pm1^{\circ}\text{C}$, $65\pm5\%$ R.H. and a photoperiod of 16:8 L:D in a growth chamber.

Data analysis

Lethal concentration value (LC_{50}) and its corresponding 95% fiducial limits (FL) for each essential oil and pesticides were esti-

mated using Polo-PC software. Lethal concentration ratios were performed to estimate the compounds' relative potency as described by Robertson *et al.* (2007). The log-probity curves were created using Microsoft Excel 2007. Data from the sublethal bioassays were analyzed using analysis of variance (ANOVA). The Duncan's Multiple Range Test separated means at 0.05 probability (SAS Institute, 2003). The percentage data were transformed to the square root of arcsine, and longevity data were transformed to square-root, before analysis.

Results

Fumigant toxicity of essential oils

The toxicity of essential oils on *T. turkestan* adults 24 h after exposure significantly increased with increasing concentrations (for *E. globulus*: $F = 14.69$; d.f. = 4, 10; $p = 0.0003$; *R. officinalis*: $F = 22.71$; d.f. = 4, 10; $p < 0.0001$; and for *M. longifolia*: $F = 5.68$; d.f. = 4, 10; $p = 0.0119$) (Figure 1). LC_{50} values of the tested essential oils are presented in Table 1. According to the LC_{50} values, *M. longifolia* was more effective than *E. globulus* and *R. officinalis* against *T. turkestan* adults (Table 1).

Contact toxicity of bioacaricides

The toxicity of acaricides significantly increased when *T. turkestan* adults were exposed to increasing concentrations of the tested compounds for 24 h (for Tondexir: $F = 12.98$; d.f. = 4, 10; $p = 0.0006$; Palizin: $F = 5.58$; d.f. = 4, 10; $p = 0.0126$; and for Spiromesifen: $F = 35.50$; d.f. = 4, 10; $p < 0.0001$) (Figure 2). The LC_{50} values of the three acaricides tested are presented in Table 2. These values and the confidence limits of toxicity ratios revealed that these acaricides' toxicity was significantly different for *T. turkestan* adults. The chemical acaricide Spiromesifen was significantly more effective than the two bioacaricides Tondexir and Palizin against *T. turkestan* adults (Table 2).

Sublethal effects

The sublethal effects of different com-

Figure 1. Log- Probit curve of plant essential oils of *Eucalyptus globulus*, *Rosmarinus officinalis* and *Mentha longifolia* on the strawberry spider mite *Tetranychus turkestan*.

Table 1. Probit analysis and lethal concentration ratios (% 95 fiducial limits) of *Eucalyptus globulus*, *Rosmarinus officinalis* and *Mentha longifolia* essential oils against the adult females of *Tetranychus turkestan*.

Parameters	Essential oil		
	<i>E. globulus</i>	<i>M. longifolia</i>	<i>R. officinalis</i>
Slope $\pm$ SE	3.01 $\pm$ 0.71	0.79 $\pm$ 0.27	1.82 $\pm$ 0.60
LC ₅₀ (95%FL) (μ l/l air)	12.50 (1.40-30.12)	4.00 (0.54-29.64)	11.52 (3.87-16.73)
χ^2 (df=3)	8.04	15.36	13.45
RP ^a	-	0.572 (0.313-1.044)	-
RP ^b	-	-	1.005 (0.606-1.665)
RP ^c	-	-	1.757 (0.871-3.541)

^a Relative potency for LC₅₀ of *M. longifolia*/ *E. globulus*

^b Relative potency for LC₅₀ of *R. officinalis*/ *E. globulus*

^c Relative potency for LC₅₀ of *R. officinalis*/ *M. longifolia*

pounds on biological parameters of *T. turkestan* adults are shown in Table 3. All tested compounds significantly reduced the fecundity ($F = 58.76$; $df = 6, 133$; $P < 0.0001$) and longevity ($F = 7.37$; $df = 6, 133$; $P < 0.0001$)

of the treated females compared with the control. The reduction in the number of eggs was significantly greater in the female mites treated with the essential oils *E. globulus*, *M. longifolia* and *R. officinalis* than

Figure 2. Log- Probit curve of three acaricides (Tondexir, Palizin, spiramesifen) on the strawberry spider mite *Tetranychus turkestani*.

Table 2. Probit analysis and lethal concentration ratios (% 95 fiducial limits) of Tondexir, Palizin and spiramesifen against the adult females of *Tetranychus turkestani*.

Parameters	Compound		
	Tondexir	Palizin	spiramesifen
Slope $\pm$ SE	2.60 $\pm$ 0.43	1.04 $\pm$ 0.23	0.52 $\pm$ 0.16
LC ₅₀ (95%FL) (ppm)	327.34 (248.02-402.91)	858.13 (538.85-1689)	10.98 (0.23-37.20)
χ^2 (df=3)	3.14	2.02	3.24
RP ^a	-	3.143 (1.798-5.494)	-
RP ^b	-	-	0.030 (0.005-0.177)
RP ^c	-	-	0.010 (0.002-0.060)

^a Relative potency for LC₅₀ of Palizin/Tondexir

^b Relative potency for LC₅₀ of spiramesifen/Tondexir

^c Relative potency for LC₅₀ of spiramesifen/Palizin

in females treated with the chemical acaricide spiramesifen. No significant difference was observed in longevity between the females treated with spiramesifen and the females treated with tested plant compounds. The percentage of egg hatchability was also

significantly affected by the treatments ($F = 3.34$; $df = 6, 35$; $P = 0.0104$). Compared with the control, the females treated with spiramesifen and three plant compounds, Palizin, *E. globulus* and *M. longifolia* had significantly lowered hatchability (Table 3).

Table 3. Sublethal effects of different compounds on *Tetranychus turkestani* adults (Mean $\pm$ SE)^a.

Treatment	Number of eggs per female	% Egg hatch	Female longevity (days)
Control	20.15 $\pm$ 1.27 a	100.00 $\pm$ 0.00 a	15.95 $\pm$ 0.68 a
Tondexir	16.10 $\pm$ 0.64 bc	82.33 $\pm$ 11.20 abc	10.45 $\pm$ 0.84 bc
Palizin	17.2 $\pm$ 0.70 b	78.66 $\pm$ 5.89 bc	12.05 $\pm$ 0.95 b
Spiromesifen	13.90 $\pm$ 0.46 c	72.16 $\pm$ 6.60 c	10.30 $\pm$ 1.05 bc
<i>Eucalyptus globulus</i>	10.3 $\pm$ 1.13 d	77.83 $\pm$ 5.64 bc	11.35 $\pm$ 1.00 b
<i>Mentha longifolia</i>	1.2 $\pm$ 0.09 e	79.28 $\pm$ 0.32 bc	7.90 $\pm$ 0.69 c
<i>Rosmarinus officinalis</i>	8.80 $\pm$ 0.88 d	88.83 $\pm$ 11.16 ab	9.70 $\pm$ 1.02 bc

^a Means in each column followed by the same letter were not significantly different at the 0.05 level when tested by Duncan's Multiple Range Test.

Discussion

Essential oils are phytochemicals with repellent and toxic properties against mites (Cavalcanti *et al.*, 2010; Sertkaya *et al.*, 2010; Attia *et al.*, 2012; Camilo *et al.*, 2017). Our results clearly showed that essential oils from the aromatic plants *R. officinalis*, *M. longifolia*, and *E. globulus* possess acaricidal activity against *T. turkestani* adults. The fumigant toxicity of *M. longifolia* was higher than that of *E. globulus* and *R. officinalis*. Furthermore, mite mortality increased with essential oil concentration. As shown in a previous study, the essential oil of *M. longifolia* is active against *T. urticae* with an LC₅₀ value of 20.08 μ l/l air (Motazedian *et al.*, 2012). Aissaoui *et al.* (2019) reported substantial contact toxicity of *E. globulus* essential oil against different stages of *T. urticae* and a continuous increase in mortality rates of spider mite larvae and adults was observed with increasing concentration. In another study, Hussein *et al.* (2013) examined the contact activity of three plant essential oils, including that of *E. globulus* against females of *T. urticae*. They found that *E. globulus* essential oil is effective with an LC₅₀ value of 2.202%. Efficacy of rosemary oil when applied as an acaricide to control two-spotted spider mite has been reported in previous studies (Miresmailli & Isman, 2006; Miresmailli *et al.* 2006). In the study conducted by Choi *et al.* (2004),

rosemary oil was not acutely toxic (mortality <60%) against *T. urticae* compared with oil of eucalyptus.

Spiromesifen is one of the most promising chemical acaricides for managing spider mites. It is an acetyl-CoA-carboxylase inhibitor, a lipid metabolism enzyme (Kontsedalov *et al.*, 2009; Sato *et al.*, 2011; Reddy & Latha, 2013). Our results suggested that spiromesifen was the most toxic acaricide to adult mites with an LC₅₀ value of 10.98 ppm, which was lower than the LC₅₀ (12.53 ppm) reported by Kumari *et al.* (2017) against *T. urticae*. Based on our results, the bioacaricides TON and PAL were toxic to adult mites. Antonious *et al.* (2007) indicated that the pesticide TON has lethal effects on *T. urticae*, which could be attributed to pepper extract as an active agent in this formulation. Our results also indicate that PAL was the least toxic (LC₅₀ = 858.13 ppm) against *T. turkestani*, i.e. 78 times less effective than spiromesifen. In another study, Mirfakhraie and Mohamadian (2017) reported the repellency effect of PAL and contact toxicity of TON against females of *T. urticae* with an LC₅₀ value of 2130.91 ppm.

Our findings revealed that all the compounds at LC₅₀ significantly affected *T. turkestani* females' biological parameters compared to control. The chemical acaricide spiromesifen and all three plant compounds caused adverse effects on *T. turkestani* fe-

males' longevity. Reduced female longevity led to a reduction in reproductive capacity. The highest significant decrease in fecundity rate by 94% was observed after exposing newly emerged adults to *M. longifolia* essential oil. Similarly to our results, Momen *et al.* (2018) reported the great potential of *M. longifolia* to deterrent the oviposition of *T. urticae* by 99.4% decrease in egg production. In the study conducted by Mohamed *et al.* (2015), the extract of *M. longifolia* reduced *T. urticae* female longevity and ovipositional period and the total number of laid eggs. Furthermore, sublethal effects of plant essential oils on the fecundity and fertility of spider mites have been reported in the previous studies (Momen *et al.*, 2001; Lim *et al.*, 2011; Abd El-Moneim *et al.*, 2012; Gholamzadeh-Chitgar *et al.*, 2013; Esmaeily *et al.*, 2017).

Hatchability of *T. turkestan* eggs was also significantly reduced by spiromesifen and some plant compounds tested, including PAL, *E. globulus* and *M. longifolia*. However, TON and *R. officinalis* did not significantly affect the percentage of eggs hatched. Similarly, Ismail *et al.* (2011) reported that *R. officinalis* oil did not affect two-spotted spider female fertility. *There are no other studies concerning the impact of TON and PAL on biological parameters of spider mites to the best of our knowledge.* The chemical acaricide spiromesifen also reduced the fecundity and egg hatchability of *T. turkestan* to 31 and 28%, respectively. Similarly, Nauen *et al.* (2005) found that spiromesifen significantly reduced the fecundity of two-spotted spider mite females at the concentrations between 0.064 and 40 mg/l Marcic *et al.* (2009) also reported that the number of laid eggs and egg hatchability of *T. urticae* was significantly affected by 180 mg/l, 18 mg/l, and 1.8 mg/l concentrations of spiromesifen.

In summary, the present study showed that *M. longifolia*, *E. globulus* and *R. officinalis* essential oils and two bioacaricides (TON and PAL) had satisfactory effects against *T. turkestan* even at a low concentration level. Given the above results and the environmentally friendly properties of plant com-

pounds, along with their low persistence in nature and low risk for humans and mammals, these compounds can be considered as reduced-risk pesticides and useful alternatives to synthetic chemicals for spider mite control. These laboratory findings need to be confirmed with further trials to investigate this bioinsecticide' sublethal effect on field populations of this pest. Furthermore, it is critical to test the safety of these compounds before practical use in *T. turkestan* control in commercial greenhouses.

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Ακαρεοκτόνος και υποθανατηφόρος δράση διαφόρων ουσιών φυτικής προέλευσης έναντι του τετράνυχου *Tetranychus turkestan* σε σύγκριση με το συνθετικό ακαρεοκτόνο spiromesifen

F. Sohrabi και M. Ziaee

Περίληψη Ο τετράνυχος *Tetranychus turkestan* Ugarov and Nikolski είναι ένας από τους κύριους εχθρούς των καλλιεργειών στο νοτιοδυτικό Ιράν και άλλες τροπικές περιοχές. Στην παρούσα εργασία εξετάστηκαν η καπνιστική δράση τριών αιθέριων ελαίων που εξήχθησαν από φυτά δενδρολίβανου (*Rosmarinus officinalis* L.), άγριας μέντας (*Mentha longifolia* L.) και ευκάλυπτου (*Eucalyptus globulus* Labill.), και η δράση επαφής δύο ακαρεοκτόνων φυτικής προέλευσης (Tondexir και Palizin) σε ενήλικα θηλυκά άτομα του *T. turkestan*, σε σύγκριση με αυτή του συνθετικού ακαρεοκτόνου spiromesifen. Επίσης, εκτιμήθηκε η υποθανατηφόρος δράση των υπό δοκιμή αιθέριων ελαίων στη συγκέντρωση LC₅₀, σε διάφορες βιολογικές παραμέτρους του τετράνυχου. Οι τιμές LC₅₀ για τα αιθέρια έλαια του ευκάλυπτου, του δενδρολίβανου και της άγριας μέντας ήταν 12,50, 11,52 και 4,00 μl/ l ατμού, ενώ για το spiromesifen, το Tondexir και το Palizin ήταν 10,98, 327,34 και 858,13 ppm, αντίστοιχα. Όλες οι δοκιμασθείσες ουσίες μείωσαν σημαντικά τη διάρκεια ζωής των ενήλικων θηλυκών ατόμων, σε ανάλογα επίπεδα με το spiromesifen. Επιπλέον, μειώθηκε η γονιμότητα σε όλες τις επεμβάσεις, με μεγαλύτερη μείωση αυτή στα αιθέρια έλαια. Το Palizin και τα αιθέρια έλαια του ευκάλυπτου και της άγριας μέντας μείωσαν σημαντικά την εκκόλαψη των ωών του τετράνυχου σε επίπεδα ανάλογα με το spiromesifen. Σύμφωνα με τα αποτελέσματα, οι δοκιμασθείσες φυτικές ουσίες είναι αποτελεσματικές έναντι του τετράνυχου *T. turkestan* και αποτελούν κατάλληλες εναλλακτικές ουσίες σε συνθετικά φυτοφάρμακα για την αντιμετώπισή του.

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