

ORIGINAL ARTICLE

Effects of water-based extracts of peppermint (*Mentha piperita* L.) and French marigold (*Tagetes patula* L.) on the transformation of larvae and nymphs of two-spotted spider mite (*Tetranychus urticae* Koch)

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Abstract

The population dynamics of *Tetranychus urticae* (two-spotted spider mite – TSSM) are influenced by environmental factors, which were investigated to identify methods limiting the development and harmfulness of this pest. This paper presents findings from a laboratory study on the effect of spraying with water-based extracts of *Mentha piperita* and *Tagetes patula* plants on the growth rate of TSSM larvae and nymphs. The time required for the transformation of the larvae into protonymphs was longer for *T. patula* water-based extracts than extracts from *M. piperita*. Differences in the time of transformation from the nymphal stage to adult TSSM were also observed depending on the plant water-based extracts, their concentration, and the host plant on which nymphs were developing. The use of 100 g · l⁻¹ French marigold extract was associated with longer transformation time of nymphs on discs of pea leaves than all tested concentrations of French marigold extract applied to sugar beet leaves. Ten percent of the extracts from peppermint plants had a stronger limiting effect on TSSM nymphs on pea leaves than on bean or sugar beet leaves.

Keywords: immature stages, interactions, *Mentha piperita*, *Tagetes patula*, *Tetranychus urticae*

Introduction

The two-spotted spider mite (TSSM) – *Tetranychus urticae* Koch (Acari: Tetranychidae), is a widespread mite (De Carvalho Ribeiro *et al.* 2019) found in all climate zones. The database of its host plants includes about 1,275 species representing 70 genera and dozens of botanical families (Migeon and Dorkeld 2021). It feeds on wild plants and crops, including vegetables, ornamental and agricultural plants, as well as fruit

trees and shrubs. Crops, including economically important species, account for about 10% of all TSSM host plants (Vacante 2016; Abad *et al.* 2019). Intensive feeding of TSSM combined with a rapid increase in its population size has a negative effect on the physiology of the whole plant and reduces yield size and quality (Nyoike and Liburd 2013). Muluken *et al.* (2016) found that TSSM caused the total destruction

of potato plants grown in a field. Nyoike and Liburd (2013) reported strawberry yield losses ranging from 50 to 80%. Ulatowska *et al.* (2015) and Jakubowska *et al.* (2018) reported up to 50% sugar beet yield losses and up to 2% decrease in sugar content, respectively.

TSSM is also harmful to sugar beet *Beta vulgaris* L. (Jakubowska *et al.* 2022). For several years, large populations of this pest have been observed in plantations in Central Poland, in the Wielkopolskie, Kujawsko-Pomorskie, Łódzkie, Lubelskie and partly Mazowieckie provinces, where sugar beet is a popular crop. This region of Poland is characterized by agrometeorological conditions favorable for the development of TSSM during the growing season (dry springs, high summer temperatures 25–30°C and low precipitation 0–200 mm) (Jakubowska *et al.* 2022).

The control of TSSM mainly relies on the use of synthetic acaricides. However, these products are not always effective because TSSM has a high potential for developing resistant populations (Nicastro *et al.* 2013). Incorrectly used acaricides can cause contamination of the environment and food, especially fruit and vegetables intended for the fresh market (Vinicius *et al.* 2018). Because of the trend towards reducing the use of synthetic plant protection products and concerns about the natural environment and its biodiversity, new effective ways to control the harmfulness of TSSM have been investigated. In this area of interest, studies are being carried out on the development of biopesticides based on natural substances and natural properties of plants, on the effects of microorganisms, their metabolites, and beneficial organisms (Jakubowska *et al.* 2022).

One method to reduce the harmfulness of pests in agriculture relies on the use of natural properties of plants. Many essential oils are used as insecticides due to their direct action, biodegradability and low mammalian toxicity (Choi *et al.* 2004; Sanches Politi *et al.* 2012; Turchen *et al.* 2016). Many experimental studies have investigated secondary plant metabolites to identify effective and safe agents protecting against spider mites (Souto *et al.* 2021). Studies on the effects of various botanical extracts on spider mites (Tetranychidae) intensified in the 1980s and are continuing. Extracts obtained from the above-ground and underground parts of plants have lethal effects on spider mites, act as repellents and inhibit oviposition (Ebadollahi *et al.* 2014; De Carvalho Ribeiro *et al.* 2016; Pavela *et al.* 2018; Premalatha *et al.* 2018; Topuz *et al.* 2018; Rincón *et al.* 2019; Bhullar *et al.* 2021; De Santana *et al.* 2021). More than 100 plant species representing many botanical families, mainly Asteraceae, have been tested so far for their effectiveness against *T. urticae* (Camilo *et al.* 2017; Agut *et al.* 2018).

When selecting potential pesticidal plant extracts, several properties must be considered, including

miticidal control at low concentrations and lack of toxicity to non-target animals, so that they can be used safely in the environment in sustainable agriculture. In addition, plant extracts often contain a mixture of active substances that can delay or prevent the development of resistance. In this context, some plant extracts toxic to herbivorous mites offer promising alternatives to chemical acaricides because they are rich sources of bioactive substances.

One of the most important plant species tested to date is French marigold – *Tagetes patula* L. (Asteraceae). *T. patula* plants produce terpenes, thiophenes and many polyacetylene compounds for which different effects have been reported: biocidal (De Souza *et al.* 2022), antibacterial, antifungal, insecticidal and nematocidal and tick-repelling (Benelli *et al.* 2016; De Souza *et al.* 2022). Ismail *et al.* (2019) reported that the *T. patula* extract had toxic, ovicidal and repellent effects on TSSM and caused a 30–50% reduction in oviposition. Extracts of *Matricaria recutita* L., *Achillea millefolium* L. strongly decreased the fecundity, viability and feeding intensity of *T. urticae* (Rincón *et al.* 2019).

Two water-based extracts, from *T. patula* and peppermint *Mentha piperita* L., were evaluated in our study for their acaricidal effect. The essential oils from plants used in the experiment contain volatile secondary metabolites, mainly terpenes (De Souza *et al.* 2022), which are botanical insecticides, and some of them are commercially available ingredients of pesticides. Many essential oils are used as insecticides due to their direct activity, biodegradability and low ecotoxicity (Turchen *et al.* 2016). In this context, the toxic effects of some medicinal plant extracts on phytophagous mites are promising substitutes for synthetic pesticides, since they are rich sources of bioactive substances (Ismail *et al.* 2019). Essential oils from peppermint, French marigold and other plants contain many natural compounds with acaricidal properties. The insecticidal and acaricidal effects of peppermint oil are attributed to terpenoids (De Souza *et al.* 2022).

The exposure of spider mites to plant extracts is expected to change the pest's life cycle, and then limit or inhibit pest development, its feeding, and minimize the negative impact on plants. From a plant protection perspective, females are the most important life stages of TSSM. Females feeding on host plants damage plant tissues, and their fecundity is a key target in attempting to control this pest's harmfulness. Juvenile larval and nymphal stages also feeding on plants account for about half of the TSSM lifespan. Therefore, it is important to investigate the effects of plant protection products on these life stages of the spider mite.

The presented experiments aimed to assess the effects of water-based extracts of peppermint and French marigold on the transformation of juvenile forms of TSSM (larvae and nymphs) on leaf discs of sugar beet

B. vulgaris, pea *Pisum sativum* L. and common bean *Phaseolus vulgaris* L.

Materials and Methods

Collection of plants and TSSM population used in the experiments

Plants of French marigold cv. Bonita Carmen and peppermint were grown in a growth chamber ($20 \pm 2^\circ\text{C}$ and photoperiod 16 h light: 8 h dark), in pots filled with soil substrate KRONEN^R mixed with gravel (1 : 1). The above-ground parts of the plants were used for the preparation of water-based extracts. The TSSM were kept at a controlled temperature ($25 \pm 2^\circ\text{C}$) and photoperiod (16 h light : 8 h dark, 16L : 8D), in a plant growth chamber on *B. vulgaris* cv. Krajana, *P. sativum* cv. Hubal, and the *P. vulgaris* cv. Livia. Discs were cut out from the leaves of each of the plants mentioned above (free of TSSM) and placed on moist cotton wool in Petri dishes. TSSM females that had begun oviposition were placed on the discs and left for 24 hours to deposit eggs. The females were removed from the discs, and the emergence of TSSM larvae was monitored. The larvae which had hatched during the last 4 hours were used for the experiment. To rear nymphs, females were placed on sugar beet, pea and bean leaf discs and left for oviposition. Females were removed, and the larvae hatching and immobilization were monitored. Nymphs that had undergone molting during the last 4 hours were used for the experiment.

Bioassays of water-based extracts of plants

Fresh above-ground parts of French marigold (cv. Carmen) and peppermint (*M. piperita*) plants were chopped and immersed in water in adequate proportions to obtain concentrations of $1 \text{ g} \cdot \text{l}^{-1}$, $10 \text{ g} \cdot \text{l}^{-1}$ and $100 \text{ g} \cdot \text{l}^{-1}$. Liquid soap (Biały Jeleń; $1 \text{ g} \cdot \text{l}^{-1}$) was added to water to improve the extraction of non-polar compounds from the plant material. The mixtures were left to stand for 24 hours at an ambient temperature ($20 \pm 5^\circ\text{C}$). After 24 h the extracts were filtered through sterile gauze, poured into dark glass bottles until used in the experiment.

Experimental design

To examine the effect of water-based extracts of French marigold and peppermint on juvenile stages of TSSM, an experiment was carried out in Petri dishes under controlled conditions ($25 \pm 2^\circ\text{C}$ with a photoperiod 16L : 8D). Discs of 1 cm^2 cut out from the leaves of sugar beet, pea and bean were placed on cotton wool

soaked in water in Petri dishes. The discs were sprayed once with water-based extracts of French marigold and peppermint, at the concentrations mentioned above, with doses of $5 \mu\text{l}$ per cm^2 of leaf surface. This experiment was performed twice (in June and September 2022), in 15 replicates for each combination. The control discs in this experiment were specimens placed on discs sprayed with water. Discs were left to dry, and one TSSM larva or protonymph was placed on each. The separate experiments were set up for larvae and nymphs. Observations were carried out for 5 days following spraying. The transformation of the juvenile stages of TSSM was determined based on the observation of the morphology of individuals and the occurrence of a molt indicating the completion of this process.

Statistical analysis

The effect of spraying with water-based extracts of French marigold and peppermint on the development of TSSM was assessed using a four-factor analysis of variance (ANOVA) to determine the effect of the kind of water-based extract, its dose, kind of plant disc and series. The assumptions necessary for the ANOVA concerning the normality of the distribution of the observed traits were verified using the Shapiro and Wilk (1965) test for normality, while the equality of variances was verified with the Levene test (Schultz 1985). An alternative method, i.e., the Cumulative Link Model (CLM) (Agresti 2010) implemented in the *R*'s *ordinal* package was used for statistics that did not meet the requirements of normality and equality. Detailed hypotheses were verified following the approach proposed by Searle (Searle *et al.* 1980) called Estimated Marginal Means (EMMs) and implemented in the *emmeans* package (Lenth 2023). Homogeneous groups were established based on this method.

All statistical analyses were performed using the R language version 4.2.2 (R Core Team 2022). A graphical presentation of statistics was prepared using the *ggplot2* package (Wickham 2016).

Results

Effects of spraying on the growth rate of TSSM

Larva

The following factors were tested in the Cumulative Link Model: dose, host plant on which the development of larvae was observed, plant from which water-based extracts were prepared, and the series of experiment. The significance of individual variables is presented in Table 1.

Table 1. The significance of individual variables tested with Cumulative Link Model in the experiment set up for larvae

	df	Pr(>Chi)
Dose	3	0.004957 **
Plant discs	2	0.434072
Water-based extract	1	1.756e-09 ***
Series	1	0.115005

Significant codes for p-value: ***0.001, **0.01, *0.05, 0.1, 1

The CLM analysis revealed that the kind of water-based extract and its dose significantly affected the development of larvae. The model with interaction showed no significant differences. The conducted experiment revealed significant differences in the duration of the larval stage for the kind of water-based extract and the doses used ($1 \text{ g} \cdot \text{l}^{-1}$ and $100 \text{ g} \cdot \text{l}^{-1}$) compared to the control. EMMs for the post-hoc test revealed that the use of *T. patula* sprays was associated with a longer larval stage during transformation into protonymph than sprays prepared from *M. piperita*. Statistics are presented in Table 2.

Nymph

The analysis of the significance of factors demonstrated that the statistics obtained for series 1 (Table 3) differed from those obtained for series 2 (Table 4). In both series, the host plant on which water-based extracts were tested, the plant discs and dose and kind of water-based extracts and dose interactions had significant effects.

Comparative analysis demonstrated significant differences between the levels of factors for 54 pairs in series 1 and for 30 pairs in series 2. Comparison of statistics for both series identified 23 common pairs.

The application of $100 \text{ g} \cdot \text{l}^{-1}$ extract of *T. patula* onto discs of pea leaves was associated with longer transformation from protonymph to the adult stage than all tested concentrations of this extract applied to discs of *B. vulgaris* leaves. The application of $100 \text{ g} \cdot \text{l}^{-1}$ *T. patula* extract on discs of pea leaves was also associated with longer development of juvenile TSSMs than $1 \text{ g} \cdot \text{l}^{-1}$ and $1 \text{ g} \cdot \text{l}^{-1}$ extracts and the control on discs of *P. sativum* and *P. vulgaris* leaves. The development of nymphs on *P. sativum* discs sprayed with $100 \text{ g} \cdot \text{l}^{-1}$ *T. patula* extract was longer than the control variant and samples treated with $1 \text{ g} \cdot \text{l}^{-1}$ and $1 \text{ g} \cdot \text{l}^{-1}$ extracts.

Table 2. Summary of multiple comparisons for the model considering the type of plant for the preparation of water-based extracts and the doses in the experiment set up for larvae

Dose	Estimate	Standard Error	z-score	p-value	Significance
0% – 0.1%	-0.7265380	0.2202138	-3.2992390	0.0053584	**
0% – 1%	-0.3611629	0.2169740	-1.6645448	0.3425997	
0% – 10%	-0.5936226	0.2186710	-2.7146835	0.0335629	*
0.1% – 1%	0.3653751	0.2206113	1.6561936	0.3471539	
0.1% – 10%	0.1329154	0.2219387	0.5988833	0.9324284	
1% – 10%	-0.2324597	0.2191779	-1.0605981	0.7135460	
Water-based extract	estimate	standard error	z-score	p-value	Significance
Tp – Mp	0.9245788	0.1561748	5.920154	0	***

Tp – *Tagetes patula*, Mp – *Mentha piperita*, Significant codes for p-value: *** 0.001, ** 0.01, * 0.05, 0.1, 1

Table 3. Significance of individual factors analyzed with the Wald Chi-squared test for series 1 in the experiment set up for nymphs

	df	Chi-square	Pr(>Chi)
Plant discs	2	33.7558	4.677e-08 ***
Water-based extract	1	0.7899	0.374116
Dose	3	12.8891	0.004883 **
Plant discs: water-based extract	2	5.5264	0.063090
Plant discs: dose	6	51.0746	2.861e-09 ***
Water-based extract: dose	3	53.7613	1.262e-11 ***
Plant discs: water-based extract: dose	6	22.0886	0.001167 **

Significant codes for p-value: ***0.001, **0.01, *0.05, 0.1, 1

Table 4. Significance of individual factors analyzed with the Wald Chi-squared test for series 2 in the experiment set up for nymphs

	df	Chi-square	Pr(>Chi)
Plant discs	2	38.3193	4.776e-09 ***
Water-based extract	1	1.0446	0.306758
Dose	3	2.7798	0.426835
Plant discs: water-based extract	2	22.4114	1.360e-05 ***
Plant discs: dose	6	47.5348	1.463e-08 ***
Water-based extract: dose	3	12.2937	0.006442 **
Plant discs: water-based extract: dose	6	6.8556	0.334404

Significant codes for *p*-value: ***0.001, **0.01, *0.05, 0.1, 1

The transition of TSSM nymphs to the adult stage was longer on *P. sativum* leaves sprayed with 100 g · l⁻¹ *T. patula* extract than nymphs developing on *B. vulgaris* and *P. vulgaris* treated with 1 g · l⁻¹ or 100 g · l⁻¹ *M. piperita* extracts. Using 100 g · l⁻¹ extracts of *M. piperita* had a more limiting effect on TSSM nymphs on *P. sativum* than on *P. vulgaris* and *B. vulgaris* leaves.

Discussion

Most studies investigating the limiting effect of natural substances on TSSM concern females, which is the most important life stage affecting the growth of TSSM populations. Kumar *et al.* (2009) reported a significant reduction in oviposition and lifespan of TSSM exposed to fumigants with 5% menthol solutions of essential oil from *M. arvensis* L. However, an experiment carried out by De Souza *et al.* (2022) revealed an increase in the fecundity of TSSM females exposed to *M. piperita* hydrodistillates and pure menthol. Menthol also has acaricidal and repellent properties, as observed by Isman and Tak (2017).

In our study we observed the limiting effects of *M. piperita* water-based extracts on the development of TSSM larvae and nymphs. The impact of *M. piperita* sprays on nymphs was stronger when 1 g · l⁻¹ and 100 g · l⁻¹ solutions were used, which may be associated with the concentrations and proportions of chemical compounds in the applied extracts used in the experiment.

Essential oils and menthol also have an almost 100% repellent effect on mosquitoes *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* when applied on human skin (Ansari *et al.* 2000), a behavior-modifying effect on *Periplaneta americana* (Jankowska *et al.* 2019), and a strongly repellent and ovicidal effect on storage pests *Callosobruchus maculatus*, *Rhyzopertha dominica*, *Sitophilus oryzae* and *Tribolium castaneum* (Aggarwal *et al.* 2001), and on larvae of *Drosophila melanogaster* that exhibited an aversive rolling response (Himmel *et al.* 2019). The effectiveness

of menthol was also demonstrated against bacteria and fungi (Kalemba and Synowiec 2020). Essential oils and menthol can be considered as potential acaricides for the control of *T. urticae* due to their toxic effect on all life stages of the pest. Currently, these substances are regarded as promising bioacaricides.

Furthermore, many studies have shown that *T. patula* exhibits antibacterial, antifungal, and insecticidal activities (Faizi *et al.* 2008; Vidal *et al.* 2009). Plants from the *Tagetes* genus, mainly *T. patula*, *T. erecta* L. and *T. minuta* L., have been extensively studied for their potential use in pest management. For example, 1% extract of aerial parts of *T. minuta* caused 50–80% mortality in TSSM females (Chermenskaya *et al.* 2010). Ismail *et al.* (2019) reported that the ethanolic extract of *T. patula* leaves had a toxic effect on TSSM. It also had an ovicidal and repellent effect on the pest and deterred oviposition by 30–50% on the discs of leaves from sweet potato *Ipomoea batatas*.

Conclusions

1. Water-based extracts of *M. piperita* and *T. patula* affect the larval and nymphal stages of *T. urticae*.
2. Larval development depends on the dose (concentration) and type of plant from which repellent water-based extracts are prepared. The growth rate of nymphs is also influenced by the host plant species colonized by TSSM.

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