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Article

Acaricidal activity of Shirazian thyme and rosemary methanolic extracts in combination with spirodiclofen and propargite on *Tetranychus urticae* (Acari: Tetranychidae)

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ABSTRACT

The two-spotted spider mite, *Tetranychus urticae* Koch is one of the polyphagous pests that attack a wide range of crops. In recent decades, excessive application of synthetic acaricides has led to its resistance to pesticides and environmental pollution. However, considering the importance of the role of pesticides in controlling plant pests, it is impossible to cease using these compounds. In recent decades, new methods such as the use of plant extracts have been proposed that not only control pests but also have no residues and harmful environmental effects. Also, the use of plant extracts in combination with synthetic pesticides while controlling the pest, reduces the amount of pesticide usage. In this study, the efficacy of methanolic extracts of *Zataria multiflora* and *Rosmarinus officinalis* was investigated individually and in combination with spirodiclofen and propargite to control two-spotted spider mites. Bioassay tests were performed using the leaf dipping method. Each treatment was performed in three independent biological replications and the mortality was recorded 24 h after exposure. The 50% lethal dose (LC_{50}) of spirodiclofen and propargite as well as methanolic extracts of *Z. multiflora* and *R. officinalis* were estimated to 1.89, 12.76, 1934.13, and 4382.07 mg/l, respectively. In addition, results of experiments related to mixing plant extracts with acaricides showed that the combination of *Z. multiflora* extract with spirodiclofen and propargite caused a synergistic effect with a co-toxicity coefficient of 66.66 and 55.55, respectively. However, the combination of *R. officinalis* extract with spirodiclofen and propargite caused an antagonistic response with −77.78 and −80.56, respectively.

KEY WORDS: Interaction; plant extracts; *Rosmarinus officinalis*; two-spotted spider mite; *Zataria multiflora*.

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INTRODUCTION

The two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is one of the most important cosmopolitan pests (Geng *et al.* 2014). *Tetranychus urticae* attacks about 1200 species of plants including many weeds, field crops, vegetables, ornamental, and greenhouse plants (Migeon *et al.* 2010). Greenhouse cucumber is one of the plants that is seriously damaged by this pest.

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Tetranychus urticae attacks host plants and reduces total chlorophyll content and the net rate of photosynthesis in the cucumber leaves, which causes leaf deformation (Park and Lee 2002). The high population of *T. urticae* causes webbing, yellow spots appearance, and finally the fall of cucumber leaves (Kumral *et al.* 2010). The main control strategies of *T. urticae* in greenhouses are related to the use of synthetic pesticides, however, their excessive use in recent years has led to adverse effects such as mite resistance, secondary pest replacement, resurgence, and residual effects of pesticides on final yield (Homayoonzadeh *et al*. 2020). Widespread use of pesticides along with high fecundity, arrhenotokous reproduction, high mutation rate, and very short life cycle has led to *T. urticae* evolving resistance to almost all class of pesticides (Akyazi *et al.* 2018). Due to the resistance of *T. urticae* to many pesticides such as organophosphate, abamectin, clofentzin, hexizetox, bifenthrin, and chlorphenipyrme, this mite can be called the "pesticide resistance champion" (Van Leeuwen *et al.* 2015).

As an alternative to synthetic pesticides, plant extracts were investigated which as a source of bioactive substances (Pontes *et al.* 2011). The properties of botanical pesticides including effectiveness against the target pest, economically valuable, easy handle and apply, with little or no residue on plants, and environmental safety are well-known (Isman 2006). Moreover, plant-based pesticides often contain a mixture of active substances, which can delay or prevent resistance development (Rattan 2010). Synergistic activity between synthetic pesticides and plant extract is a powerful tool for the development of an efficient and more eco-friendly pest control strategy (B-Bernard and Philogène 1993).

Numerous studies have examined the effects of plant extracts from different species belonging to the Lamiaceae family on the *T. urticae*. The results have shown acaricidal, ovicidal, repellency, inhibition of oviposition, and anti-nutritional effects on the mite (Rincón *et al.* 2019). *Zataria multiflora* Boissier and *Rosmarinus officinalis* L. used in this study are from Lamiaceae family and we assumed that they have acaricidal activity against *T. urticae*.

In this study, methanolic extracts of *Z. multiflora* and *R. officinalis* were used alone and in combination with spirodiclofen and propargite to control two-spotted spider mites. The goal of this study is to use plant extracts to minimize the problems caused by synthetic pesticide application. The hypothesis of this study is that plant extracts in combination with acaricides can reduce the dose usage of acaricides against *T. urticae.* To prove this hypothesis, bioassays and calculation of LC_{50} of *Z. multiflora* and *R. officinalis* were performed alone and in combination with spirodiclofen and propargite.

MATERIALS AND METHODS

Plant material and growth conditions

Cucumber seeds (*Cucumis sativus* cultivar superN3, Hed, USA) were planted and grown in 15-cm-diameter plastic pots filled with sterilized planting mix consisting of cocopeat and perlite (1:1). Plants were grown in a greenhouse under climate-controlled conditions of 25 ± 2 °C, 50 ± 5% RH, and 16L: 8D photoperiod.

Mite colony

The colony of *T. urticae* was established from the mite colony of insect population dynamics laboratory at the University of Tehran. Mites were reared on cucumber seedlings in growth chambers set to 25 ± 2 °C, 50 ± 5% RH, and 16L: 8D photoperiod.

Chemicals

Spirodiclofen (Envidor®, SC 240؛ Bayer CropScience, Germany) and propargite (Omite®, EC 57%; Ariashimi Agrochemicals Formulator, Iran), were used in this study. Tween® 80 (polysorbate, Merck, Germany) and Triton® X–100 (for analysis, Merck, Germany) were also used as adjuvants in this experiment.

Preparation of plants crude extracts

Zataria multiflora B. was collected from Rangelands of Shiraz in September 2020, and *Rosmarinus officinalis* L. was collected from the Campus of the University of Tehran, Karaj in September 2020. Collected plants were identified by plant biologists at the University of Tehran. Preparation of plant extracts was carried out using the method of Geng *et al.* (2014). Leaves of plants were dried at room temperature and powdered with an electric blender. Finally, 50 g of plant sample powder was dissolved in 250 ml of methanol (Neutro USP Pure®, Lab Reagent, Iran) and left static for 24h at dark conditions. After 24 h, solutions were filtered through a Buchner funnel under vacuum pressure. To obtain the crude extract, the filtered solutions were concentrated to the dryness point with a rotary evaporator (Heidolph, VV 1, Germany) at 40 ℃ for 20 min. The obtained crude extract was stored at 4 ℃ under dark conditions for subsequent experiments.

Bioassay

The toxicity of plant extracts and synthetic acaricides was evaluated in pre-tests using various concentrations to establish the five concentrations for final analysis. Final concentrations used in the bioassay were 1, 1.7, 3.1, 5.6, and 10 ppm for spirodiclofen; 1, 4.7, 22.3, 104.7, and 500 ppm for propargite; 500, 1006, 2009, 4008, and 8000 ppm for Shirazian thyme methanolic extract; 1000, 1778, 3162, 5623, and 10000 ppm for rosemary methanolic extract. The leaf dip method of Paramasivam and Selvi (2017) was used in bioassay analysis to obtain 50% lethal concentration (LC_{50}). Leaf discs (2.5-cm diameter) were prepared from cucumber plant leaves and dipped in acaricides solutions in distilled water or mathanolic plant extract solutions for five seconds, then left to dry at room temperature and placed upside down on wet cotton in a petri dish (3-cm diameter). Distilled water was used as control. Twenty female adult mites (24 h old) were transferred on cucumber leaf discs, and after 24 hours of treatment, the mortality rate was recorded. Failing in mites' responses after probing with fine brush was considered as dead. All experiments were carried out in three independent biological replications.

Synergistic effects

For the study of synergistic effects, the LC_{30} of *Z. multiflora*, *R. officinalis*, Spirodiclofen, and propargite on female adult *T. urticae* was determined. Then a solution with a ratio of (1: 1 v/v) was prepared from each of these extracts with each of the mentioned acaricides alone and in combination with 0.01% of adjuvants (Tween® 80 and Triton® X-100). Twenty female adult mites (24 h old) were exposed to each of the toxic solutions according to the previous section and the mortality rates were recorded 24 hours post treatment. Co-toxicity coefficient (CTC) was calculated in order to determine the magnitude of change in the efficacy of each acaricides occurring in combination with plant extracts. CTC was calculated using the following equation 1:

$$\text{Equation 1: CTC} = \frac{\%\,observed\ mortality - \%\,Expected\ mortality}{\%\,Expected\ mortality} \times 100$$

Where, CTC > 1 and CTC < 0 show synergistic and antagonistic interactions, respectively, and 0 < CTC < 20 show additive effect (Sun and Johnson 1960).

RESULTS

Acaricidal activity of plant extracts and acaricides on female adults

The toxicity of plant extracts, as well as synthetic acaricides against *T. urticae* have been presented in Table 1. The results show that both extracts may have acaricidal properties but *T. utricae* is more sensitive to *Z. multiflora* extract than the other one. Also, the most lethal effect is related to spirodiclofen with the lowest LC_{50}.

Table 1. Toxicity of *Z. multiflora*, *R. officinalis*, spirodiclofen and propargite on adult females of two-spotted spider mites.

Source	Number of mites tested	LC_{50}(mg/l) CI (95%)	Slope ± SE	χ^2 (df)	Heterogeneity
Z. multiflora	360	1934.137	1.291 ± 0.885	9.589 (13)	0.738
R. officinalis	360	4382.074	1.312 ± 0.873	12.606 (13)	0.970
Spirodiclofen	360	1.896	2.805 ± 0.298	4.922 (13)	0.379
Propargite	360	12.768	1.030 ± 0.103	8.366 (13)	0.644

Df = degrees of freedom, χ 2 = chi-square, CI = confidence intervals.

Effects of mixing Z. multiflora *extract with spirodiclofen and propargite on adult females of* T. urticae

According to the CTC, which is higher than 20 for all treatments, results well demonstrated the synergistic effect in all treatments obtained from the combination of Shirazian thyme extract and acaricides (Table 2). According to the results, the combination of *Z. multiflora* extract with spirodiclofen and propargite, even without the use of any adjuvants, had a synergistic effect.

Table 2. The effect of mixing *Zataria multiflora* extract with spirodiclofen and propargite on mortality percent of *T. urticae* adult females.

Mixture	%Mortality		CTC
	Expected	Observed	
Z. multiflora **+ Spirodiclofen**	60	100	66.66
Z. multiflora **+ Spirodiclofen +Tween80**	60	100	66.66
Z. multiflora **+ Spirodiclofen +Triton X-100**	60	96.66	61.10
Z. multiflora **+ Propargite**	60	93.33	55.55
Z. multiflora **+ Propargite + Tween80**	60	100	66.66
Z. multiflora **+ Propargite + Triton X-100**	60	88.33	47.22

Table 3. The effect of mixing *Rosmarinus officinalis* extract with spirodiclofen and propargite on adult females of two-spotted spider mites.

Mixture	%Mortality		CTC
	Expected	Observed	
R. officinalis **+ Spirodiclofen**	60	13.33	−77.78
R. officinalis **+ Spirodiclofen + Tween80**	60	36.66	−38.90
R. officinalis **+ Spirodiclofen + Triton X-100**	60	23.33	−61.11
R. officinalis **+ Propargite**	60	11.66	−80.56
R. officinalis **+ Propargite + Tween80**	60	31.66	−47.23
R. officinalis **+ Propargite + Triton X-100**	60	23.33	−61.11

Effects of mixing R. officinalis *extract with spirodiclofen and propargite on adult females of* T. urticae

According to the CTC, which is less than one for all treatments, we found an antagonistic effect in all treatments obtained from the combination of *R. officinalis* extract and the tested acaricides. According to Table 3, the combination of *R. officinalis* extract with spirodiclofen and propargite, even without the use of any adjuvants, had an antagonistic effect.

DISCUSSION

Numerous studies have examined the effects of plant-derived compounds from different plant families, including Lamiaceae on insects and mites (Rincón *et al.* 2019). A large number of essential oils obtained from this family has been produced commercially. The compounds extracted from this family mainly include monoterpenoids and diterpenoids, which have various biological activities against arthropods that has made them a potential source of insecticides and acaricides (Yorulmaz *et al.* 2014). Most research has examined the effects of plants extracted from this family on mites, for example, Rasikari *et al.* (2005) carried out a screening of the leaf extracts of 67 species of plants belonging to the Lamiaceae. They found that plant extracts from the plants *Clerodendrum traceyi*, *Premna serratifolia*, *Ceratanthus longicornis* and *Plectranthus habrophyllus* caused a 100% mortality on *T. urticae* and *Gmelina leichardtii*, *Premna acuminata*, *Viticipremna queenslandica*, *Plectranthus diversus*, *Plectranthus glabriflorus* and *Plectranthus suaveolens* caused mortality between 90% and 99% on *T. urticae*. In this study, the lethal effect of extracts of two species of the Lamiaceae family, including *Z. multiflora* and *R. officinalis* on the *T. urticae* was investigated and results have shown the lethal effects of methanolic extracts of these plants on *T. urticae*.

In various studies, the effects of plant extracts of both species on mites or insects have been studied. Razavi *et al.* (2015) demonstrated that methanolic extracts of *Z. multiflora* and *Lepidium latifolium* cause 86.26% and 100% losses on *Varroa destructor* mite populations, respectively. However, both extracts had little effect on bees and therefore they can be used to control varroa mites in beehives. Numerous experiments have been performed on *R. officinalis* extracts against various pests and different results have been obtained from the effects of rosemary extract against pests. Salman *et al.* (2018) found that rosemary extracts at a concentration of 12% at six days after the treatment caused 79% and 62% mortality on protonymph and deutonymph of *T. urticae*, respectively. Also, mortality percentage in males and females of *T. urticae* in response to rosemary extract was 58% and 82%, respectively. Nevertheless, the mortality percentage of rosemary extract at one day after treatment was 24.6% in adults and 14.8% in nymphs of *T. urticae*. Meanwhile, rosemary extract caused 82.2% of the losses on the eggs of *T. urticae* (Salman *et al.* 2018). The effect of rosemary extract on predatory mites including *Phytoseiulus persimilis* and *Neoseiulus californicus* was also investigated by Salman *et al.* (2018). They showed that rosemary extract caused 33.3% and 38.4% mortalities on mentioned predatory mites, respectively. A number of studies have investigated the different effects of *R. officinalis* essential oil on *T. urticae*. Salman *et al.* (2014) investigated the effect of essential oils of four species of Lamiaceae including rosemary, lavender, sage, hyssop on different growth stages of *T. urticae*. The results showed that all essential oils had a repellent and lethal effect against nymphs and adult mites. Also, rosemary essential oil had the highest contact toxicity effect on adults at 96 hours after treatment compared to other essential oils. In another study, Miresmailli and Isman (2006) illustrated that rosemary essential oil is effective on spider mites and also has no adverse effect on host plants at two days after treatment. The lethal effects of plant extracts are related to the compounds contained in them, including alkaloids, limonoids, terpenoids, flavonoids and phenolic and tannin (Rao and Nath 2005). Therefore, it can be noted that the difference in the effectiveness of *Z. multiflora* and *R. officinalis* extract in this study may be due to different amounts of these compounds in the plant leaves extraction.

It seems that using a combination of plant extracts with synthetic acaricides is an effective way to control mites. Because in addition to making an effective controlling agent in pest management, it reduces the use of pesticides and also reduces the rate of resistance development. Synergistic formulations may be more bioactive than individual pesticides against different pests. A lot more work has been done on the synergistic activity of synthetic-synthetic pesticides than plant-plant and plant-synthetic pesticide combinations against various herbivorous pests (B-Bernard and Philogène 1993). In another part of the present study, the efficacy of plant extracts along with spirodiclofen and propargite on *T. urticae* was investigated. The results showed that mixing *Z. multiflora* extract with both acaricides caused a synergistic response while mixing *R. officinalis* extract and acaricides caused an antagonistic effect. Propargite is a sulfite ester and its effect is to disrupt the oxidative phosphorylation process. Spirodiclofen is also one of the most important compounds in the tetronic acid group, which has a disruptive effect on the biosynthetic cycle of lipids by inhibiting the action of acetyl-CoA carboxylase (Nauen 2005). It seems that there are relations to changes in the detoxifying enzymes of mites with synergistic/antagonistic effects. Perhaps, biochemical impacts of plant extracts on herbivores' detoxification systems causes an increase or decrease in the efficiency of acaricides. In other words, activation or inhibition of detoxifying enzymes through plant extracts causes synergistic/antagonistic effects and also results in changes in mortality percentages. Meanwhile, it is well-known that plant extracts have many different chemical groups in their structure. Thus, it seems that the main reason for the synergistic and antagonistic effect in mixing *Z. multiflora* and *R. officinalis* extracts with acaricides may be related to chemical compatibility between them. In chemical compatibility, the components of the mixture do not chemically react together and also cause an increase in pest control (Hink and Feel 1986). A synergistic effect was observed in a mixture of methanolic extract of *Z. multiflora* with spirodiclofen and propargite that caused increased mortality on *T. urticae*. However, in a mixture of *R. officinalis* methanolic extract with mentioned acaricides, it seems that the chemical reaction between components of the extract and acaricides may reduce the mortality percent of two-spotted spider mites and illustrated an antagonistic response.

CONCLUSION

The results of this study showed that Shirazian thyme methanolic extract in combination with spirodiclofen and propargite has a synergistic effect. However, rosemary methanolic extract in combination with mentioned acaricides has an antagonistic effect. Therefore, according to the results, it can be recommended to use the methanolic extract of *Zataria multiflora* in combination with spirodiclofen and propargite in *T. urticae* control in the cucumber greenhouse.

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فعالیت کنه‌کشی عصارۀ متانولی آویشن شیرازی و رزماری در ترکیب با اسپیرودیکلوفن و پروپارژیت بر روی کنۀ تارتن دولکه‌ای، *Tetranychus urticae* (Acari: Tetranychidae)

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چکیده

کنۀ تارتن دولکه‌ای، *Tetranychus urticae* Koch یکی از آفات مهم و چندخوار است که بازۀ گسترده‌ای از محصولات کشاورزی را مورد حمله قرار می‌دهد. در دهه‌های اخیر استفادۀ بیش از حد از آفتکش‌های مصنوعی، بروز مقاومت در آفات و آلودگی‌های زیست محیطی را به همراه داشته است. اما با توجه به اهمیت نقش آفتکش‌ها در کنترل آفات حذف این ترکیبات ممکن نیست. امروزه روش‌های جدیدی از جمله استفاده از عصاره‌های گیاهی ارایه شده است که نه تنها سبب کنترل آفات می‌شود بلکه فاقد باقیمانده و آثار مخرب زیست محیطی است. همچنین استفاده از عصاره‌های گیاهی در ترکیب با آفتکش‌های مصنوعی ضمن کنترل آفت، میزان استفاده از آفتکش مصنوعی را کاهش می‌دهد. در این پژوهش کارایی عصاره‌های متانولی آویشن شیرازی (*Zataria multiflora*) و رزماری (*Rosmarinus officinalis*) به تنهایی و همراه با اسپیرودیکلوفن و پروپارژیت برای کنترل کنه‌های تارتن دولکه‌ای بررسی شد. آزمایش‌های زیست‌سنجی به روش غوطه‌وری برگ انجام شد. هر یک از تیمارها در چهار تکرار انجام و میزان مرگ و میر ۲٤ ساعت پس از تیمار شدن ثبت شد. LC_{50} اسپیرودیکلوفن و پروپارژیت و همچنین عصاره‌های متانولی آویشن شیرازی و رزماری به ترتیب ۱/۸۹٦، ۱۲/۷٦۸، ۱۹۳٤/۱۳۷ و ٤۳۸۲/۰۷٤ میلی‌گرم بر لیتر محاسبه شد. همچنین نتایج آزمایش‌های مربوط به اختلاط عصاره‌های گیاهی با آفتکش‌ها نشان داد مخلوط عصارۀ آویشن شیرازی همراه با اسپیرودیکلوفن و پروپارژیت به ترتیب با ضریب سمیت مشترک ٦٦/٦٦ و ٥٥/٥٥ دارای اثر سینرژیستی‌اند. در حالی‌که مخلوط عصارۀ رزماری همراه با اسپیرودیکلوفن به ترتیب با ضریب سمیت مشترک ۷۷/۷۸ – و ٥٦/ ۸۰– اثر آنتاگونیستی دارد.

واژگان کلیدی: برهم‌کنش؛ عصارۀ گیاهی؛ رزماری؛ کنۀ تارتن دولکه‌ای؛ آویشن شیرازی.

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