Evaluation of chemical acaricides against the two-spotted spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae) in geothermal greenhouses in southern Tunisia

El Habib Gaid¹,², Sabrine Chouikhi*,¹,², Besma Assadi¹,³, Kameleddine Nagaz¹, Kaouther Lebdi Grissa⁴ & Mohamed Sadok Belkadhi¹,³

¹Drylands and Oases Cropping Laboratory Arid Lands Institute, 4119 Medenine, Tunisia
²Faculty of Sciences of Gabes, University of Gabes, Street Riadh, Zrig, 6072, Gabes, Tunisia
³Technical Center for Protected and Geothermal Cultures (CTCPG), Gabes, Tunisia
⁴Institut National Agronomique de Tunisie, 43 Avenue Charles Nicolle, 1082, Cité Mahrajène, Tunis, Tunisia

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*Corresponding author
chouikhi.sabrine22@gmail.com

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1. INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae), is a major threat to geothermal greenhouses in southern Tunisia, causing severe damage to cucurbit and solanaceous crops (Chouikhi et al., 2022). In the field, the use of acaricides for chemical control of spider mites has become the predominant method in integrated management programs, mainly due to its ease of application and low cost (Bernardi et al., 2013). However, the frequent use of various chemicals has led to a significant increase in the problem of multi-resistance to acaricides in these mites, presenting a major challenge to their effective management (Osakabe et al., 2009). The emergence of resistance to acaricides means that higher doses of commercial products need to be applied to maintain their effectiveness against mites. This practice, however, increases the ecological risks associated with their use. It is therefore imperative to develop new acaricides, with innovative modes of action and different structural characteristics, to combat phytophagous mites effectively and sustainably (Yu et al., 2019).

Cyflumetofen, whose chemical formula is [2-methoxyethyl (R, S)-2-(4-tert-butylphenyl)-2-cyano-3-oxo-3-(α, α, α-trifluoro-O-tolyl)-propionate], is the first acaricide in the complex II inhibitor class, acting on the mitochondrial electron transport chain (MET). Initially introduced in Japan, this compound belongs to the new class of benzoylacetonitrile-based acaricides, and effectively targets all stages of development of various economically important mites, including the genera *Tetranychus* and...
Panonychus (Hayashi et al., 2013; Takahashi et al., 2012; Wei et al., 2019).

(E)-Cinnamaldehyde, a phenylpropanoid compound, is a major constituent of essential oils extracted from plants of the Cinnamomum genus. This compound exhibits a range of biological activities, including antifungal (Shreaz et al., 2016) and antibacterial properties (Chang et al., 2008), as well as efficacy as an insecticide (Cheng et al., 2009). In addition, (E)-Cinnamaldehyde has shown significant acaricidal activity in various studies (Novato et al., 2015), underlining its potential in the fight against harmful mites.

Fenazaquine is one of a number of acaricides and insecticides that appear to act by inhibiting NADH: ubiquinone oxidoreductase (complex I) in the concentration range from 1 to 10 nM. It is remarkably effective on contact against tetranychid and eriophid mites, both in the laboratory and in the field (Shanker et al., 2001). Its mechanism of action lies in complex I of the mitochondrial respiratory chain, where it acts as an inhibitor of electron transport (Yaqoob et al., 2021).

Pyridaben, a derivative of pyridazinone, is an insecticide and acaricide approved for use in the EU and several other countries around the world. The compound affects metabolism, inhibiting the electron transport chain in mitochondria by binding to complex I at the coenzyme Q0 site. It works exactly by blocking mitochondria and preventing oxidation of the isolated high-resistance complex I (Dekeyser, 2005). This product acts by inhibiting the oxidoreductase activity of NADH:CoQ, and is effective against all stages of development of various mite species (Stumpf and Nauen, 2001).

Abamectin is a reduced-risk neuroactive acaricide insecticide (chloride channel activator). It is a mixture of natural products, avermectin B1a (>80%) and avermectin B1b (<20%), isolated from the fermentation of the soil bacterium Streptomyces avermitilis. Abamectin is considered safe for beneficial arthropods under field conditions due to its short persistence in the environment, rapid uptake by treated plants and rapid degradation of surface residues (Krämer and Schirmer, 2007).

Spinosad is an insecticide derived from a fermentation product of the soil-borne actinomycete Saccharopolyspora spinosa. It is toxic by ingestion and contact, and has a unique mode of action on the insect nervous system (Van Leeuwen et al., 2005).

The aim of this study was to evaluate the efficacy of six acaricides: Cyflumetofen 193.5 g/kg SC, Cinnamaldehyde w/w: 22.03 EC, Fenazaquin 20% SC, Pyridaben 200 g/kg WP, Abamectin 3% + Spinosad 2% EW, and Diafenthiuron, against T. urticae infesting tomato crops under heated greenhouses in southern Tunisia.

2. MATERIAL AND METHODS

2.1. Experimental site

The trial was carried out in a greenhouse growing tomatoes (Solanum lycopersicum) of the “Maria” variety, naturally infested by T. urticae (Fig. 1). This is an early crop planted in October in a 500 m2 geothermal monotunnel greenhouse, containing 4 rows 60 m long, run in conventional mode. The chosen greenhouse is in full production and no insecticide treatments were applied during the trial.

Fig. 1. Female and eggs of Tetranychus urticae

2.2. Treatments

The efficacy of five acaricides tested against spider mites in a tomato greenhouse was evaluated against an untreated control and a Pegasus reference acaricide based on Diafenthion. Treatments were carried out using a 16 L backpack sprayer (Table 1). Each individual plot was treated with an acaricide, avoiding the edges.

2.3. Experimental protocol

Trials were conducted using a completely randomized block design. A line of tomato crops represented each block. The block was divided into seven 7-meter-long elementary plots. During treatments, the plots were isolated from each other by a 2m/2m Plexiglas plate. Within the blocks, the individual plots were each treated with an acaricide according to a pre-established scheme (Fig. 2).
2.4. Sampling

The sampling was carried out five times over a 15-day period, on days 0, 3, 7, 10 and 15. For each elementary plot, 40 leaves were taken at random from the different plants. These samples were stored in plastic bags at 4°C before being examined under a binocular magnifying glass. During this examination, the number of eggs, larvae, protonymphs, deutonymphs and live adult males and females is counted on each leaf.

2.5. Statistical analysis

The degree of efficacy of each treatment against the different stages of *T. urticae* on tomato leaves was assessed by applying the population reduction rate according to Abbott’s formula (Abbott, 1925)

\[
TR\% = \left(\frac{P1 - P2}{P1}\right) \times 100
\]

where:
- \(P1\) = Mite density in control block.
- \(P2\) = Density of mites in the treated block.
- \(TR\%\) = the rate of reduction in leaf mite population level at a given date after treatment.

Statistical analyses were performed using IBM SPSS Statistics 22 software.

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**Table 1. List of acaricides tested against *Tetranychus urticae***

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Active ingredient</th>
<th>Chemical family</th>
<th>IRAC classification</th>
<th>Doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Untreated control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>Cyflumetofen 193.5g/kg SC</td>
<td>β-Ketonitrile derivatives</td>
<td>25</td>
<td>1 l/ha</td>
</tr>
<tr>
<td>T3</td>
<td>Cinnamaldehyde w/w: 22.03 EC</td>
<td>phenylpropanoids</td>
<td>4</td>
<td>250 cc/hl</td>
</tr>
<tr>
<td>T4</td>
<td>Fenazaquin 20% SC</td>
<td>METI insecticides (Mitochondrial Electron Transport Inhibitors)</td>
<td>21A</td>
<td>50 cc/hl</td>
</tr>
<tr>
<td>T5</td>
<td>Pyridaben 00g/kg WP</td>
<td></td>
<td>21A</td>
<td>100 g/100 l</td>
</tr>
<tr>
<td>T6</td>
<td>Abamectin 3%+Spinosad 2% EW</td>
<td>Avermectin + Spinosyn</td>
<td>6 + 5</td>
<td>75 cc/hl</td>
</tr>
<tr>
<td>T7</td>
<td>Diaphenthiuron</td>
<td>Carbamidimide precursors</td>
<td>12A</td>
<td>1 l/ha</td>
</tr>
</tbody>
</table>

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**Fig. 2. Experimental protocol diagram**
3. RESULTS AND DISCUSSION

In the tomato greenhouse, the acaricidal effect of treatments based on Cyflumetofen, Cinnamaldehyde, Fenazaquin, Pyradaben and a combination of Abamectin 3% and Spinosad 2% compared with that of the reference treatment Diafenthiuron and that of an untreated control is based on calculating the rate of reduction of eggs, larvae, deutonymphs, protonymphs, females and males of the spider mite.

3.1. Effect of acaricides on *T. urticae* eggs

The efficacy of five treatments based on Cyflumetofen, Cinnamaldehyde, Fenazaquin, Pyradaben and a combination of Abamectin 3% and Spinosad 2% was evaluated and compared with that of the reference treatment, Diafenthiuron, in controlling *T. urticae* eggs. Reduction rates, calculated in relation to the untreated control, are shown in Fig. 3. Three days after treatment, a reduction in eggs ranging from 85.76% to 93.63% was observed. From day 7 onwards, the substances tested showed comparable efficacy to the reference treatment, with reduction rates in excess of 99.33%. Statistical analysis showed no significant difference between the substances tested and the reference substance (F=1; P=0.423) 15 days after treatment.

3.2. Effect of acaricides on *T. urticae* larvae

As with the eggs, the various treatments resulted in a significant reduction in *T. urticae* larvae, as shown in Fig. 4. The Cinnamaldehyde treatment was particularly effective, reducing the number of *T. urticae* larvae by 97.40% on the third day after treatment. At the last sampling date, according to statistical analysis, treatments with Cyflumetofen, Cinnamaldehyde, Pyradaben, a combination of Abamectin 3% and Spinosad 2%, as well as Diafenthiuron, all resulted in

![Fig. 3. Eggs reduction rate of Tetranychus urticae](image)

![Fig. 4. Larvae reduction rate of Tetranychus urticae](image)
significant reductions in the number of *T. urticae* larvae, with rates ranging from 97.53% to 100%. In contrast, the fenazaquin treatment was less effective, with a larval reduction rate of 89.50%, demonstrating a significant difference from the other treatments (F=4.601; P=0.001).

### 3.3. Effect of acaricides on *T. urticae* protonymphs

The efficacy of five treatments - Cyflumetofen, Cinnamaldehyde, Fenazaquin, Pyradaben and a combination of Abamectin 3% and Spinosad 2% - in controlling *T. urticae* protonymphs is compared with that of the reference treatment, Diazinon, in Fig. 5. Three days after application, these treatments all showed significantly high efficacy, with protonymph population reduction rates in excess of 84.40%, surpassing the reference treatment. Subsequently, a general increase in reduction rates was observed. However, 15 days after treatment, Fenazaquin showed slightly diminished efficacy, with a protonymph reduction rate of 91.02%, lower than that of the other treatments (F= 3.478; P=0.007).

### 3.4. Effect of acaricides on *T. urticae* deutonymphs

As with *T. urticae* protonymphs, all five tested acaricides demonstrated significant effectiveness against deutonymphae, achieving reduction rates exceeding 90.76%. By the final sampling date, the reduction percentages for *T. urticae* deutonymphae reached 100% for treatments based on Cyflumetofen, Cinnamaldehyde, Fenazaquin, Pyradaben, and the combination of Abamectin and Spinosad. In comparison, the reference treatment, Diazinon, achieved a 96.54% reduction (Fig. 6). Statistical analysis revealed no significant difference between them in the efficacy of these substances in reducing *T. urticae* deutonymphs (F=1.000; P=0.423).

![Fig. 5. Protonymphs reduction rate of *Tetranychus urticae*](image1)

![Fig. 6. Deutonymphs reduction rate of *Tetranychus urticae*](image2)
3.5. Effect of acaricides on *T. urticae* females

The reduction in female *T. urticae* populations following acaricide application is shown in Fig. 7. Each treatment induced a significant reduction in the number of females, with rates ranging from 90.76% to 100% as early as three days after application. Analysis of variance of the reduction rates reveals that there was no significant difference between the results obtained with treatments based on Cyflumetofen, Cinnamaldehyde, Fenazaquin, Pyradaben, the combination of Abamectin and Spinosad, and those of the reference treatment, Diafenthiuron (F= 0.797; P= 0.555).

Fig. 7. Females reduction rate of *Tetranychus urticae*

3.6. Effect of acaricides on *T. urticae* males

Throughout the follow-up period, the different acaricide substances produced significant reductions in male *T. urticae* populations, as well as in the other developmental stages of this mite. Analysis of variance of reduction rates of male *T. urticae* showed no significant difference between the treatments tested and the reference treatment. Reduction percentages ranged from 96.55% to 100% (Fig. 8). However, the fenazaquin-based treatment proved less effective, with a male reduction rate of 91.02%, indicating a significant difference from the other treatments (F= 3.49; P= 0.006).

Fig. 8. Males reduction rate of *Tetranychus urticae*
In this trial, the five-acaricide products tested, Cyflumetofen, Cinnamaldehyde, Fenazaquin, Pyridaben, and a combination of Abamectin 3% and Spinosad 2%, showed significant efficacy in reducing egg populations and motile forms of *T. urticae* in a tomato greenhouse. This efficacy was comparable to that of the reference treatment based on Diamfenthiuron. Several studies have demonstrated the efficacy of these acaricides. Cyflumetofen proved highly effective against most spider mite populations (İnak et al., 2019). Cyflumetofen has proved effective against several species of *Tetranychus urticae* (two-spotted spider mite), *Tetranychus kanzawai* (Kanzawa spider mite) and *Panonychus citri* (red citrus mite) that have developed resistance to currently available acaricides (Khajehali et al., 2011). Cyflumetofen is highly effective against both *P. citri* and *T. urticae* (Hayashi et al., 2013). The results of a study revealed that cyflumetofen (Danisaraba®) had sublethal effects on *T. urticae* life history parameters and could have an effect on population development (Mokhtari et al., 2022). Cyflumetofen showed low toxicity to the predatory mite *Phytoseiulus persimilis*, with mortality below 35% at the highest concentration tested (Han et al., 2024). In addition, the integrated application of predator and acaricide demonstrated control efficacy in excess of 95% after 28 days, suggesting its promising potential for the management of spider mites in greenhouse strawberry crops (Han et al., 2024). Similarly to our results, one study demonstrated that cinnamaldehyde has a significant acaricidal effect. Application of cinnamaldehyde at concentrations of 500, 1000, 1500, 2000, 2500 and 3000 ppm against the two species tested revealed maximum mortality of 90.84% and 93.64% respectively for two mites infesting date palms in Saudi Arabia, namely *Oligonychus australis* (McGregor) and *Eutetranychus palmatus* Attiah (Alhewairini et al., 2021).

In addition, fenazaquin was found to be highly effective against *T. urticae* and slightly harmful to the predatory mite *Neoseiulus californicus* (Urbaneja et al., 2008). A study showed that fenazaquin, applied at doses of 125 and 150 g ma/ha, significantly reduced the population of *T. urticae* on tomato, with a 92.13% reduction leading to significantly higher fruit yields of 30.0 to 32.1% compared with the control. In addition, fenazaquin proved harmless to predatory phytoseiid mites at all doses tested (Misra, 2011). In another study on *T. urticae* infestation on eggplant, treatment with 0.01% fenazaquine 10 EC proved highly effective, recording the lowest mite density per leaf (0.80 mites/leaf) (Shukla and Radadia, 2018). Laboratory bioassays were carried out to assess the relative toxicity of fenazaquine 10 EC against the rice mite, *Oligonychus oryzae*. Fenazaquine proved effective, recording 100% mortality of pregnant females 24 hours after treatment application. Egg mortality was 86.60% after 72 hours (Aswin et al., 2015). In addition, fenazaquine and abamectin showed significant efficacy against two-spotted spider mites, generating up to 100% mortality in the laboratory. Both acaricides completely eliminated mites 48 hours after treatment (Reddy et al., 2014).

In our study, the combination Abamectin 3%+Spinosad 2% EW applied at a dose of 75 cc/l showed a significant reduction in the *T. urticae* population in greenhouse tomatoes. It should be noted that several studies examined the efficacy of each substance individually. One study evaluated the efficacy of Abamectin at a concentration of 0.50 ml/l, demonstrating high mortality rates, reaching 94.0%, in motile forms of *T. urticae* after 72 hours of application under laboratory conditions (Hanash et al., 2020). Similarly, (Ismail et al., 2007) reported that Vapcomic 1.8% EC (a formulation of abamectin) resulted in 87% mortality of *T. urticae* eggs at a concentration of 2.5 mg/L. Furthermore, abamectin showed the highest acaricidal activity against adult female *T. urticae*, followed by chlorfenapyr, while pyridaben was a significantly less toxic compound (Badawy et al., 2022). The application of abamectin and pyridaben had a significant impact on the biological parameters of *T. urticae*, including development time, survival rate and fecundity. Treated females showed a significant decrease in net reproductive rate (R0), finite growth rate (λ) and intrinsic growth rate (r) (Sabier et al., 2018). Likewise, (Villanueva and Walgenbach, 2006) showed significant results regarding the acaricidal effects of spinosad on *T. urticae* females, as well as on the reduction of oviposition.

### 4. CONCLUSION

In conclusion, this study demonstrates the efficacy of several acaricides, such as Cyflumetofen, Cinnamaldehyde, Fenazaquin, Pyridaben and Abamectin + Spinosad, in managing the population of *T. urticae* in greenhouse tomato crops. These results offer promising prospects for effective control of this species of mite pest in heated

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greenhouse crops in southern Tunisia. In addition to their effectiveness, these products help address resistance issues by belonging to diverse chemical families.

REFERENCES


