



Toxicity of Some Pesticides against *Oligonychus afrasiaticus* (Mcgregor) (Acari: Tetranychidae) in Tunisian Oases and Analysis of Their Residues in Date Fruits

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ABSTRACT

Background: In Tunisia, the date palm mite *Oligonychus afrasiaticus* McGregor (Acarina: Tetranychidae) is one of the most important pests of the date palm *Phoenix dactylifera* L. (Arecaceae). The control of *Oligonychus afrasiaticus* in Tunisian oases requires the use of safe and effective acaricides.

Methods: In this study, the toxicity of pesticides (sulfur, hexythiazox, bifentazate and spinosad) against *O. afrasiaticus* in date palms was investigated in the field. The aim of this study was also to evaluate the dissipation of bifentazate, hexythiazox and spinosad in date fruits by the QuEChERS method.

Result: Bifentazate was effective with a reduction of 90.2 and 89.4% of *O. afrasiaticus* motile forms and eggs, respectively, up to 21 days after application of this insecticide. Within 21 days of spraying, hexythiazox was an effective acaricide, reducing eggs and motile forms to 98.5% and 82.5%, respectively. Spinosad was effective for up to 14 days. Under the conditions of the Tunisian oases, the sulfur toxicity applied in July may remain effective for <14 days after treatment. Regarding, the dissipation of tested pesticides, the results showed that bifentazate deposited faster in date fruits than hexythiazox and spinosad. The initial deposition of bifentazate and spinosad decreased and was no longer detected after 7 and 14 days, respectively. Hexythiazox was detected at harvest when the date fruits were treated twice, but the residues were lower than the residue levels recommended by the European Union. These results would be useful to improve the control of *O. afrasiaticus* based on the efficacy, persistence and toxicity of these pesticides.

Key words: Dissipation, *Oligonychus afrasiaticus*, Pesticides, Residue, Toxicity.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) sector plays an important role in Tunisia. The production of dates is estimated at 340 thousand tons for 2021-2022 (Onagri, 2023). Tunisia is the largest exporter of dates in the world in terms of value (GIFruits, 2022). There is a growing demand on the international market for dates of excellent quality that are free from pesticide residues. However, dates are affected by numerous diseases and pests that affect their yield and quality, such as *Oligonychus afrasiaticus*. Farmers tend to use more acaricides to control this pest. In Saudi Arabia Al-Doghairi (2004) evaluated the efficacy of eight acaricides such as Neoron (Bromopropylate 25% EC), Kelthane (Dicofol 18.5 EC), Tedion (Tetradifon 75.2 g L⁻¹), Top cop (73% S + 6.4% Cu, w/v), Microthiol special (Sulfur 80%WP), Ekatin (Thiometon 25% EC), Transact (Abamectin 1.8% EC) and Peropal (Azocyclotin 25% WP). Aldosari (2009) evaluated the efficacy of the botanical compound (Biaco) as compared with some acaricides such as Vertamic 1.8% EC, Salocide 40% EC, Amitraz 20% EC and Perpol 25% WP. In Iran, Arbabi *et al.* (2017) evaluated the efficacy of different acaricides such as Amitraz 20% EC, Fenpropathrin 10% EC, Fenpyroximate 5% SC, Fenazaquin 20% SC, Propargite 57% EC, Tetradifon 18.5% EC, Hexythiazox 10%. In Egypt Fakeer and Eraky

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(2019) tested five acaricides abamectin (1.8% EC), chlorfenapyr (36% SC), fenpyroximate (5% EC), cyflumetofen (20% SC) and hexythiazox (10% WP).

The excessive and improper use of these agrochemicals can lead to an increase in pesticide residues in may leach

in the soil, water and even remain in the crop residues and food products. From there they enter in human food chain posing serious health hazards (Padaliya *et al.* 2020).

Pesticide exposure in people can happen both directly and indirectly. Pesticides can immediately damage the skin, eyes, mouth and respiratory system, for example, when they are sprayed on crops (Sangha *et al.* 2023; Sahoo *et al.* 2024).

For this reason, pesticide residues in food are strictly regulated by governments around the world to determine whether the concentration of pesticides used exceeds the maximum residue limits (MRLs) set by the Codex Alimentarius (CA) and the European Union (EU) (EFSA, 2021). Studies have detected pesticide residues in marketed dates, namely in Saudi Arabia in 2018 (Abdallah *et al.* 2018) and in the United Arab Emirates (UAE) in 2020 (El-Mageed *et al.* 2022). In addition, compliance with pesticide regulations and safety certification are crucial for market access. Due to this reason, many pesticides are banned in several countries, including key export markets such as Europe, which is an important export market for Tunisian products.

To avoid health risks for consumers, it is therefore important to consider the effects of the pesticide on date fruits when searching for pesticides that effectively control *O. afrasiaticus* in the Tunisian oasis. Therefore, the aim of this work was (1) to evaluate the toxicity of pesticides (sulfur, hexythiazox, bifentazate and spinosad) against *O. afrasiaticus* and (1) to measure the residue levels of bifentazate, hexythiazox and spinosad after different intervals of last field application. The results will be useful to establish a list of recommended registered acaricides for use on Tunisian date palms in Tunisia.

MATERIALS AND METHODS

Acaricides/Insecticides

The aim of this insecticide trial was to evaluate the effectiveness of commercial pesticides, hexythiazox (cesar 10 WP, Nippon Soda CO Ltd, Tokyo, Japan), spinosad (Tracer 240 SC Dow Agrosciences USA Spinosad, 240 g/l, SC) at a rate of, bifentazate (Floramite 240 SC CHEMTURA/ ARYSTA, Japon) and sulfur (azumo) were purchased from local suppliers.

Field application of insecticides

The field trial was conducted from July to November 2021 in the Chekmou oasis (3359023.7300 N, 814049.7900 E, Tozeur, southern Tunisia). It is a modern type of oasis with an average planting distance of 10 × 10 m and ten-year-old date palms (Deglet Noor variety).

The experiment has a total of Five treatments (one water control and for pesticides) and each treatment was replicated 3 times in a randomized complete block design (replicate size: three palms for each treatment).

Each test plot was sprayed on 30/7/2021 with the active ingredient at the recommended dosage: 50 g ha⁻¹ hexythiazox, 300 g ha⁻¹ sulfur, 60 mL/100 L spinosad and 60 mL/100 L bifentazate.

Sampling mites on fruit

From each replicate, a sample of 10 fruits was taken from each date palm (total of 30 fruits per replicate). A total of 90 fruits per treatment were sampled before treatment and 3, 7, 14 and 21 days after treatment.

The mites in the fruits were counted under a stereo microscope. We determined the average number of eggs and motile forms of *O. afrasiaticus* per sampling date. The efficacy of each pesticide against *O. afrasiaticus* was calculated using the equation (Henderson and Tilton, 1955).

Reduction% =

$$\left(1 - \frac{n \text{ in Co before treatment} * n \text{ in T after treatment}}{n \text{ in Co after treatment} * n \text{ in T before treatment}}\right) * 100$$

Where,

n = Number of motile stages or eggs.

T = Treated.

Co = Control.

Residues

Application of pesticides

To evaluate the persistence of bifentazate, hexythiazox and spinosad in date fruits, test plots were selected (the residue of sulfur is not investigated as it is biological). In the first plot, the date palms were sprayed once on 30/7/2021. In the second plot, the date palms were sprayed twice, on 30/7/2021 and 1/9/2021. In each plot, the experimental design was a randomized complete block with three treatments (three pesticides), three blocks and three palms (Deglet Noor variety) in each plot for each treatment.

Date samples collection and preparation

Approximately 1 kg of date palm fruits were randomly collected from each tree on 7 and 14 days after insecticide/acaricide treatment and at harvest on 29/9/2021. The seeds were discarded and the pulp was homogenized with a blender and stored at -20°C until analysis.

Analysis of residues

The "Quick, Easy, Cheap, Effective, Rugged and Safe" (QuEChERS) method was used to detect residues of spinosad, hexythiazox and bifentazate in palm fabrics (Attia *et al.*, 2019). The samples were analyzed at the Laboratory for the Control and Analysis of Pesticides (LCAP) of the Ministry of Agriculture in Tunisia. It uses dispersive solid phase extraction (SPE) followed by chromatographic analysis of the extracts (Solomon *et al.*, 1993).

Each sample was thawed naturally, then 10 g of each homogenized sample was mixed with 10 mL of acetonitrile in a centrifuge tube. After this step, a mixture of salts: 4 g MgSO₄, 1 g NaCl, 1 g sodium citrate, dihydrate (C₆H₅Na₃O₇ · 2H₂O) and 0.5 g disodium hydrogen citrate sesquihydrate (C₆H₈Na₂O₈) were added to each sample. After vigorous shaking of the mixture, the tubes were then centrifuged at 3500 rpm for 5 minutes. Six ml of the supernatant was collected and added to a tube containing 150 mg of primary

and secondary amines (PSA) and 900 mg of MgSO_4 . After stirring, the mixture was centrifuged at 3500 rpm for 5 minutes. The final extract was filtered and analyzed.

Chromatographic analyses

The analyses of pesticide residues in dates fruits were performed by a chromatographic liquid system coupled to a triple quadrupole mass spectrometer and interface by electrospray (LC-ESI-MS/MS) (Table 1).

Statistical analysis

Significant differences in efficacy of pesticides treatments were analyzed using one-way analysis of variance (ANOVA) and the means were compared using Tukey test at $P < 0.05$. All the analyses were performed using the SPSS statistical software version 21.0.

RESULTS AND DISCUSSION

The efficacy of pesticides for control of *O. afrasiaticus*

The efficacy of each pesticide in reducing the motile stages of *O. afrasiaticus* on date fruit was evaluated under field conditions (Table 2). Three days after treatment, the average number of motile stages of *O. afrasiaticus* was reported as 6.3, 6, 10.6 and 0 for bifentazate, hexythiazox, spinosad and sulfur, respectively. This corresponded to a percentage reduction in the mite population of 66.9, 68.7, 44.9 and 100 %, respectively.

By 21 days post-treatment, the average number of motile stages increased to 25, 45, 203 and 348.3 for the bifentazate, hexythiazox, spinosad and sulfur treatments, respectively. The percentage reductions showed that bifentazate (90.2 %) and hexythiazox (82.5 %) were the most effective acaricides. Spinosad led to a population reduction of 21.4%, while sulfur had no effect in reducing the population. As far as the ovicidal effect is concerned (Table 3), 19 eggs were counted three days after treatment, which means a reduction of 28 % in the fruits treated with sulfur. In fruits treated with hexythiazox, bifentazate and spinosad, egg counts were 0.6, 3.6 and 4.02, respectively. The greatest reduction in eggs was observed in fruits treated with hexythiazox (97 %), followed by bifentazate (86.6 %) and spinosad (84.9 %), with no significant differences in efficacy between the latter three treatments.

Three weeks after treatment, the number of *O. afrasiaticus* eggs on date fruit was found to be 33.6, 4.6, 311 and 308.3 for bifentazate, hexythiazox, spinosad and sulfur, respectively. The highest reduction of eggs (98.5 %) was observed in fruits treated with hexythiazox. Bifentazate also showed a significant ovicidal effect, leading to an 89.4 % reduction in eggs. In contrast, the fruits treated with spinosad and sulfur only showed a reduction of 1.7 % and 2.7 % respectively, which did not differ statistically from the control group.

Table 1: Experimental conditions of LC/MS analyses to detect residues of insecticides in dates fruits.

Chromatographic liquid (LC)	
Column	Inertsil ODS-3 (L=100 mm*2.1 mm*3 μm) (L=100 mm $\times$ ϕ =2.1 mm $\times$ 1.9 μm)
Mobile phase flow	0.2 ml/min
Temperature oven/ column	40°C
Mobile phase	5 mmol. L ⁻¹ (ammonium acetate/water, V/V)
Injection volume	10 μl
Mass-spectrometry (MS-MS)	
Nebulizer gas flow-rate	3 L/min
Drying gas flow-rate	15 L/min
Vaporizer temperature	400°C
Nebulizer gas argon	Argon of 230 KPa

Table 2: Efficacy of bifentazate, hexythiazox, spinosad and sulfur on numbers of *O. afrasiaticus* motile stages on date fruits before and after 3, 7, 14 and 21 days of spraying of pesticides.

Tested pesticides	Mean number of motile stages mites recorded/ 30 dates ($\pm$ SE)				
	Pre-treatment	Post-treatment (days)			
		3	7	14	21
Bifentazate	1355 $\pm$ 15 ^a	6.3 $\pm$ 0.3 ^a	6.6 $\pm$ 0.3 ^a	17.6 $\pm$ 0.8 ^a	25 $\pm$ 2.1 ^a
Hexythiazox	1367.6 $\pm$ 9.7 ^a	6 $\pm$ 1.1 ^a	4 $\pm$ 0.57 ^{ab}	46 $\pm$ 3.2 ^b	45 $\pm$ 3.1 ^a
Spinosad	1369 $\pm$ 4.3 ^a	10.6 $\pm$ 0.6 ^b	0.3 $\pm$ 0.3 ^b	17.6 $\pm$ 1.5 ^a	203 $\pm$ 3.51 ^b
Sulfur	1390.6 $\pm$ 2.1 ^a	0 $\pm$ ^c	14.33 $\pm$ 0.8 ^c	196.6 $\pm$ 2.8 ^c	348.3 $\pm$ 9.2 ^c
Control	1373.3 $\pm$ 2.8 ^a	19.3 $\pm$ 0.8 ^d	87.3 $\pm$ 2.7 ^d	767.6 $\pm$ 7.5 ^d	259 $\pm$ 5.6 ^d
	$F_{4,10}=2.3$; $P=0.126$)	$F_{4,10}=2.3$; $P<0.05$)	$F_{4,10}=762.4$; $P<0.05$)	$F_{4,10}=6639$; $P<0.05$)	$F_{4,10}=674.9$; $P<0.05$)

Values within the same column with a common letter (s) are not significantly different ($P < 0.05$, Tukey test).

Bifenazate showed high efficacy in killing *O. afrasiaticus*. These results are consistent with previous observations that bifenazate has significant toxicity potential for the treatment of other Tetranychidae such as *Panoncus citri* (Wang *et al.* 2021) and *Tetranychus urticae* (Li *et al.*, 2017), is toxic to leaf mites at all life stages (Van Leeuwen *et al.*, 2007). The long duration of action of bifenazate makes it an acaricide for mite control around the world (Ochiai *et al.*, 2007).

Hexythiazox provides effective control of *O. afrasiaticus* within 21 days of application. A similar high efficacy against *O. afrasiaticus* on date fruit was reported by Arbabi *et al.* (2017) and Fakeer *et al.* (2019). Negm *et al.* (2015) also observed that hexythiazox remained toxic to *O. pratensis* on date fruit for up to three weeks.

Spinosad was effective for up to 14 days. Ismail *et al.* (2007) considered that spinosad had a strong ovicidal effect on *T. urticae* eggs. Several studies have also found significant toxicity of spinosad to larvae and adult females of *T. urticae* on leaf disks in laboratory bioassays (Van Leeuwen *et al.* 2005; Villanueva and Walgenbach 2006; Ismail *et al.* 2007). Rabbi *et al.* (2021) indicated a significant egg mortality by spinosad, which was 71%. We chose spinosad because we wanted to find out whether spinosad, which is normally used to control *E. ceratoniae*, could also indirectly control *O. afrasiaticus*. Previous studies have shown that spinosad is not intended for use against spider

mites, but their populations are sometimes exposed to unintended effects, especially in greenhouses where the pesticide is commonly used against thrips, leafminers and caterpillars (Jones *et al.*, 2005; Dripps *et al.*, 2011; Santis *et al.*, 2012).

The results of this study showed that the sulfur applied in July is no longer effective from the 14th day after treatment under the conditions of the Tunisian oases. Al-Doghairi. (2004) found that the effect of sulfur (80% WP) against *O. afrasiaticus* was excellent until the 35th day after treatment. The existing literature contains contradictory statements about the effect of sulfur on phytophagous mites. There is no clear pattern as the species studied, the sulfur formulations, the dosage applied and the environmental conditions vary (Kreiter, 1987, Auger *et al.*, 2003, Costello 2003, Beers *et al.* 2009).

Analysis of residues

The changes in residue concentrations detected in date fruit over time and the European Union (EU) MRLs for bifenazate, hexythiazox and spinosad are shown in Table 4. In a single application of hexythiazox (plot 1), hexythiazox residues were not detectable at harvest. In second plot, which received two applications at harvest, the residue concentration was 0.44 and thus below the EU maximum residue level (2 mg kg⁻¹). For straw berries, the half-life of hexythiazox residues was 2.23 days (Shalaby *et al.* 2022) and 6.8 days for peaches (Guo *et al.* 2012).

Table 3: Efficacy of bifenazate, hexythiazox, spinosad and sulfur on numbers of *O. afrasiaticus* eggs on date fruits before and after 3, 7, 14 and 21 days of spraying of pesticides.

Tested pesticides	Mean number of eggs recorded/30 dates (±SE)				
	Pre-treatment	Post-treatment (days)			
		3	7	14	21
Bifenazate	1143.6±3.9 ^a	3.6±1.2 ^a	11.6±1.2 ^a	22.3± 0.8 ^a	33.6±2.3 ^a
Hexythiazox	1120.6±4.8 ^a	0.6±0.6 ^a	0±0 ^b	25±2.6 ^a	4.6±1.45 ^b
Spinosad	1132.6±4.3 ^a	4.02±1 ^a	2±0.5 ^{ab}	16.3±1.2 ^a	311±6.24 ^c
Sulfur	1134±3 ^a	19±0.57 ^b	77.6±7.2 ^c	324.6± 5.5 ^b	308.3± 19 ^c
Control	1147.3±10.9 ^a	27±2.07 ^c	70±3.6 ^c	733,3± 8.3 ^c	320,6±4.5 ^c
	$F_{4,10}=2.9$; ($P=0.077$)	$F_{4,10}=229.1$; ($P<0.05$)	$F_{4,10}=294.6$; ($P<0.05$)	$F_{4,10}=4460.8$; ($P<0.05$)	$F_{4,10}=2352.5$; ($P<0.05$)

Values within the same column with a common letter (s) are not significantly different ($P<0.05$, Tukey test).

Table 4: Bifenazate, hexythiazox and spinosad residue levels (mg kg⁻¹) detected in date fruits at different intervals (days after treatment DAT).

Pesticides	LDmg/kg	MRL -UE	Orchard 1			Orchard 2		
			Average residue± SE (mg/kg), N=3			Average residue ± SE (mg/kg), N=3		
			7 DAT	14 DAT	At harvest	7 DAT	14 DAT	At harvest
Hexythiazox	0.01	2.0	0.141±0.05	0.068±0.047	< LD	0.39±0.12	0.37±20.05	0.44±0.04 ^b
Spinosade	0.01	0.02	0.217±0.06	ND (< LD)	< LD	0.235±0.053	< LD	< LD
Bifenazate	0.01	0.02	< LD	< LD	< LD	< LD	< LD	< LD

MRL: Maximum residue limits European Union (EU).

ND: Not detected.

LD: Limit Declaration.

In bifenazate, rapid degradation began after 7 days. Studies have shown that the equivalent half-life of bifenazate in tomato fruit is 1.86 (Shalaby *et al.*, 2022), Mahmoud *et al.* (2014) found that bifenazate residues on strawberries had a degradation rate of 93 per cent at eight days after application.

For spinosad, no spinosad residues were detectable at two weeks after application and were thus far below the maximum residue levels for date fruit in the EU (0.02 mg kg^{-1}). The residues of spinosad on Siwy dates in Egypt, applied at a dose of 0.2 ml/L on date fruits before storage, were measured after 28 days of treatment. The residue data obtained showed that the residues of spinosad amounted to 0.19 mg/kg (Rashed *et al.*, 2018). In strawberry fruits, the half-life of spinosad in the fruits was 15 days after spraying (Shalaby *et al.*, 2022).

CONCLUSION

The results of this study showed that, under the conditions of the Tunisian oases, the sulfur applied in July was toxic from the 14th day onwards. The high toxicity of bifenazate on eggs and motile forms of *O. afrasiaticus* up to 21 days after application of this acaricide. The residues of bifenazate on date fruits volatilized within 7 days. The hexythiazox can provide effective control of eggs and motile forms of *O. afrasiaticus* up to 21 days after application. With a single application of Hexythiazox, residues on date fruit gradually declined within 14 days. At harvest, no more Hexythiazox residues were detectable. After two applications of Hexythiazox, the residue concentration at harvest was 0.44 and thus below the EU maximum level (2 mg kg^{-1}). Spinosad was effective for up to 14 days. No spinosad residues were found in date fruits after 7 days of treatment. These results would be helpful in developing a new strategy to control *O. afrasiaticus* in terms of reducing costs and residual effects.

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Disclosure statement

The authors confirm no relevant financial or non-financial competing interests to report.

Conflict of interest

The authors have no conflicts of interest to declare.

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