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Efficacy of *Metarhizium anisopliae* Biopesticide Compared with Two Chitin Synthesis Inhibitors Hexythiazox and Etoxazole in *Tetranychus urticae* Koch Management

Doaa F. El Sherif; Ayat, M. El-Sayed and Sherin H.M. Safar

Plant Protection Department, Faculty of Agriculture, Fayoum University, Fayoum 63514,
Egypt

*E. Mail : dfs00@fayoum.edu.eg

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ABSTRACT

The biopesticide of *Metarhizium anisopliae* and two chitin synthetic inhibitors, Etoxazole and Hexythiazox, were utilized at recommended doses for comparative purposes. Our investigation illustrates their effect on the reduction of *Tetranychus urticae* population and biological aspects. The obtained results indicated that *M. anisopliae* is capable of reducing the mite stages by a high percentage after one day of treatment (71.81, 72.02 and 69.87) for eggs, immatures and adults, respectively. The hatchability % was 0.6, 9.99, 98.8, and 99.3% on etoxazole, hexythiazox, *M. anisopliae* treatments and control, respectively. Adulticidal efficacy was evaluated; incubation periods for both sexes were prolonged with all treated pesticides. A highly significant decline in fecundity in females was observed when using hexythiazox and *M. anisopliae* (42.5 and 35.3 egg/female). The present study provides practical data on their effectiveness in reducing *T. urticae* populations as agents eco-friendly for mite management. Moreover, it was found that *M. anisopliae* biopesticide was better at reducing mite populations and female fecundity than etoxazole and hexythiazox. Our investigation confirmed the effectiveness of etoxazole and hexythiazox as ovicides.

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch, is an agricultural pest that is widespread and feeds on a variety of host plant species. (Xie *et al.*, 2006). More than 140 families and 1100 host plant types were targeted by mites (Souza-Pimentel *et al.*, 2018). It is known to attack about 1,200 different plant species. Spider mites' economic threshold was determined to be between three and more motile forms per leaf (Warabieda, 2015). The two-spotted spider mite can quickly achieve population levels that are harmful in comfortable factors due to their fast developmental rate, short generation period, and high net reproductive rate (Skorupska, 2004). Numerous acaricides have proven effective against mites and reduce their negative impacts on plant growth (Kumari *et al.*, 2017; Abou El-Ela 2014). Unfortunately, the two-spotted spider mite is rapidly gaining resistance to various pesticides due to its rapid reproduction, the appearance of several generations on the plant, the presence of different stages simultaneously, and the repetitive application of insecticides with the exact mechanisms of action. Organic chemicals that have novel action mechanisms and, are selective for pests, are also safe for both humans and the environment. Among the procedures in approaches for managing resistance is to rotate pesticides with different modes

of action. Insect growth regulators (IGRs) are pesticides that disrupt insects' normal development process and chitin production. Utilization of IGR for pest control programs for crops is expected. They had no negative impact on non-target organisms compared to traditional insecticides (Li *et al.*, 2014; Sáenz-de-Cabezón *et al.*, 2002; Sanatgar *et al.*, 2011). Additionally, entomopathogenic fungi are useful in pest management and have been shown to infect two hundred species of pests (Zimmermann, 2007). Etoxazole and hexythiazox are acaricides that target mites and ticks. They inhibit chitin synthesis, which is necessary for forming the exoskeleton during molting (Nauen and Smagghe 2006; Li *et al.*, 2014; Salman and Ay 2013).

Etoxazole is a chemical that is known as 2,4-diphenyl-1,3-oxazolines. Spider mites can be controlled by using etoxazole in various crops (Cloyd *et al.*, 2009; NIU *et al.*, 2014), effectively controlling spider mites' eggs, larvae, and nymphs. In contrast, the ability of etoxazole to combat adult spider mites is not confirmed (Suzuki *et al.*, 2002; Nauen and Smagghe, 2006). Hexythiazox belongs to the thiazolidine chemical group and has the properties ovicidal, larvicidal, and nymphicidal (Salem and AL-Antary, 2012). Adult pests are not affected by hexythiazox treatment, but the eggs laid by treated females do not develop properly (Sanatgar *et al.*, 2011). The mode of action of hexythiazox and etoxazole is to inhibit chitin synthesis, which is necessary for the formation of the exoskeleton during moulting (Nauen and Smagghe 2006; Li *et al.*, 2014; Salman and Ay, 2013).

The *Metarhizium* is a genus of entomopathogenic fungi from the Clavicipitaceae family, which is a naturally occurring soil fungus widely used as a biopesticide. It is eco-friendly and safe because it leaves no hazardous environmental residues. (Luz *et al.*, 2019; Yamamoto *et al.*, 2020; Kim *et al.*, 2020). Several reports have shown that *Metarhizium anisopliae* is used to control the two-spotted spider mite (Keno *et al.*, 2022; Bugeme *et al.*, 2015). The action of *M. anisopliae* on arthropods involves the adhesion of *M. anisopliae* spores to their host's epicuticle. Secreting proteins that include subtilisins, trypsins, chymotrypsins, and carboxypeptidases to degrade the arthropods procuticle's protein before colonizing the haemolymph. Moreover, toxin production harms tissue and ultimately results in death. The *M. anisopliae*'s ability to infect so many distinct hosts are due to its ability to produce unique types and quantities of proteins for each host (Aw and Hue, 2017).

This study aimed to comparison of two chitin synthetic inhibitors (etoxazole, hexythiazox) and the biopesticide (*Metarhizium anisopliae*). We examined their influence on the development, fecundity and egg hatchability percentage of *T. urticae*.

MATERIALS AND METHODS

Mite Rearing:

Stock colony of mites was reared at the laboratory of Plant Protection Department, Faculty of Agriculture, Fayoum University, Fayoum, Egypt. Two-spotted spider mite was reared for several generations according to the methods mentioned by (Gotoh 1997; Pontier *et al.*, 2001; Safar *et al.*, 2022)

The Pesticides Used:

The pesticides employed in this study represent products that were bought at the local market and are labeled against various crop pests. In contrast to conventional pesticides, they are also environmentally friendly and selective. The recommended field rates on labels used for the tested pesticide rates. Pesticide descriptions and rates tested are shown in (Table 1).

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

Acaricide (Common Name)	Trade name and Formulation*	Manufacturer/suppliers	Recommended Dose
Etoxazole	Dagrozom10% SC	Tarim San. Ve Tic. Turkey	25g / 100L
Hexythiazox	Macomite 10% WP	Nippon Soda Company, Japanese	20g / 100L
<i>Metarhizium anisopliae</i> (1x10 ⁸ CFU* s/gm)	Biometa 2.5% WP	Plant Protection Research Institute., Egypt.	250g / 100L

***Formulation** WP: Wettable Powder; **SC**: Suspension Concentrate

***CFU**: The Colony Forming Unit.

Mite Population Reduction Measurement:

Pepper seedlings *Capsicum annuum* L. (Solanaceae) were planted in 15 x 20 cm pots at the laboratory of Plant Protection, Faculty of Agriculture, Fayoum University, in the conditions of 22–28°C and 60±10% RH. Four separate groups of 3 pots (6 plants) each were covered with a fine net cloth to protect plants from natural infestation. All groups were infested by *T. urticae* adult females after 60 days of planting. The 1st group was treated with etoxazole (Etoxazole, 25g/ 100L of water), the 2nd group was treated with hexythiazox (Hexythiazox, 20g/ 100L of water), and the 3rd group was treated with *M. anisopliae* (*Metarhizium anisopliae*, 250g/ 100L of water) while the 4th group was untreated control sprayed with water only. A hand sprayer with one nozzle was used for foliar treatment application to whole pepper plants. Ten days after infestation, three randomly selected one-inch-square leaves were collected from each group of plants. The number of eggs and all movable stages of *T. urticae* were counted immediately before treatment and after one, three-, and seven-days post-treatment using a stereomicroscope. Calculation from (Henderson and Tilton 1955) was utilized to compute the percentage reductions in mite stages; significant changes between means were also evaluated using multiple tests at the 5% level in various treatments (Duncan, 1955).

Adulticidal Efficacy Assessment Test:

Ten newly emerged *T. urticae* adult females were transmitted to the upper surface of leaf discs of *A. wilkesiana* (2 cm diameter each) and then replicated four times. Leaf discs were kept on a moist cotton pad in a petri dish (9 cm diameter). Each dish was sprayed with one of the tested pesticides at the recommended dose in addition to the untreated control which was sprayed with water only.

Twenty-four hours after treatment, the females were examined, and dead ones were removed. It is acceptable for live females to lay eggs. To investigate the effects of tested pesticides on the biological aspects of the *T. urticae* generation, fifteen newly hatched larvae (ten for females and five for males) were reared individually on sixty rearing units that were divided into four equal groups (three pesticides and a control) Following their adult emergence, some males were kept apart to measure their longevity, while the other males copulated with the females to maintain the longevity and fecundity of the offspring.

Ovicidal Efficacy Assessment Test:

The spraying method was used to examine the ovicidal efficiency of the insecticides under investigation. Ten adult females of *T. urticae* were placed on a disk and duplicated three times. The females were removed after 24 h, the freshly laid eggs were counted, and sprayed using 1 ml of each pesticide at the recommended dose (Table 1). Water was applied to the control. The treated eggs were incubated, and then the hatchability percent of the eggs was determined. Daily, fifteen newly hatched larvae were examined (10 for females and 5 for males). The biological effects on the resulting mite generation of two pesticides (hexythiazox and *M. anisopliae*) were evaluated compared to untreated eggs.

Statistical Analysis:

The percentage reduction calculated by Henderson and Tilton (1955) in different mite stages, significant changes between means were also evaluated using multiple tests at a 5% level (Duncan 1955). Biological aspects data of *T. urticae* were analyzed using One-Way ANOVA (SPSS Version 21), where the p-value is *p-value < 0.05, **p-value < 0.01.

RESULTS AND DISCUSSION

Acaricides Effectiveness on Mite Populations:

The data indicates that hexythiazox, etoxazole, and *M. anisopliae* effectively decrease the two-spotted spider mite populations on pepper plants. Figures 1, 2, and 3. showed that all tested pesticides significantly affect every stage of *T. urticae*. As shown in Figure 1, after one and three days of treatment, the obtained results indicated that *M. anisopliae* biopesticide was the most effective at reducing egg percentage, followed by etoxazole and hexythiazox. While there were no significant differences between them after 7 days of treatment, exhibiting 92.3, 91.77 and 84.78 %, respectively. Meanwhile, a percentage reduction of 71.81, 42.33 and 24.17% and 82.54, 66.82 and 50.81% was obtained after 1 and 3 days, respectively.

In Figure 2, the reducing immatures showed no significant differences between *M. anisopliae* and hexythiazox after 1 and 3 days but presented significant differences from etoxazole. According to the initial effect, the biopesticide of *M. anisopliae* has a high reduction as the most effective treatment. Where, the reduction percentages were recorded (72.02, 60.61, and 31.75%), on *M. anisopliae*, hexythiazox and etoxazole, respectively. On the other hand, after 7 days, no significant differences appeared between the three tested pesticides.

Data in Figure 3, illustrated the adult female reduction and showed that *M. anisopliae* biopesticide after one- and three days post-treatment was a top performer, it recorded 69.87 % and 80.16 %, respectively. Meanwhile, after 7 days, it was the least effective. Etoxazole and hexythiazox caused the highest level of reduction after 7 days post-treatment (100% and 73.98) while *M. anisopliae* caused a 54.18% decrease in females.

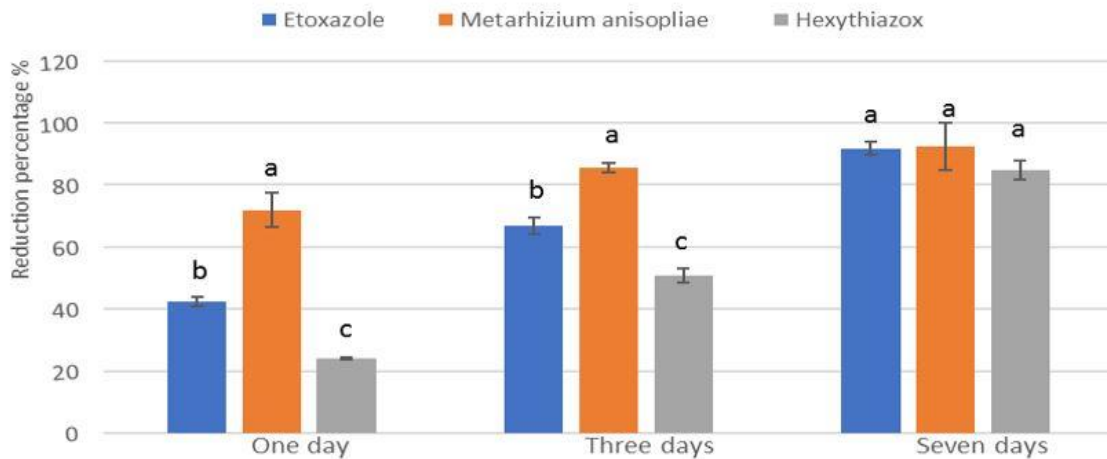


Fig 1. Reduction percentage of *T. urticae* number of eggs on pepper plants after 1, 3, and 7 days of application.

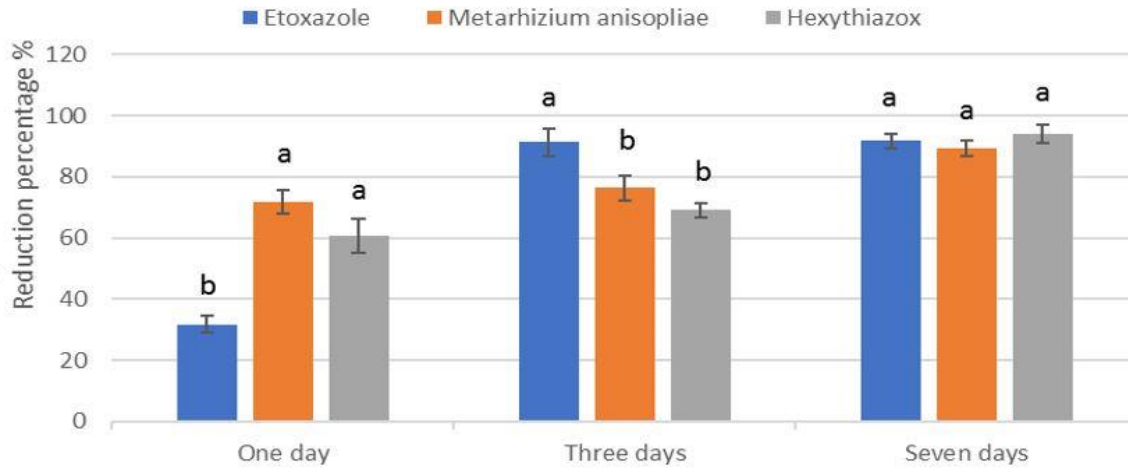


Fig 2. Reduction percentage of *T. urticae* immatures on pepper plants after 1, 3, and 7 days of application.

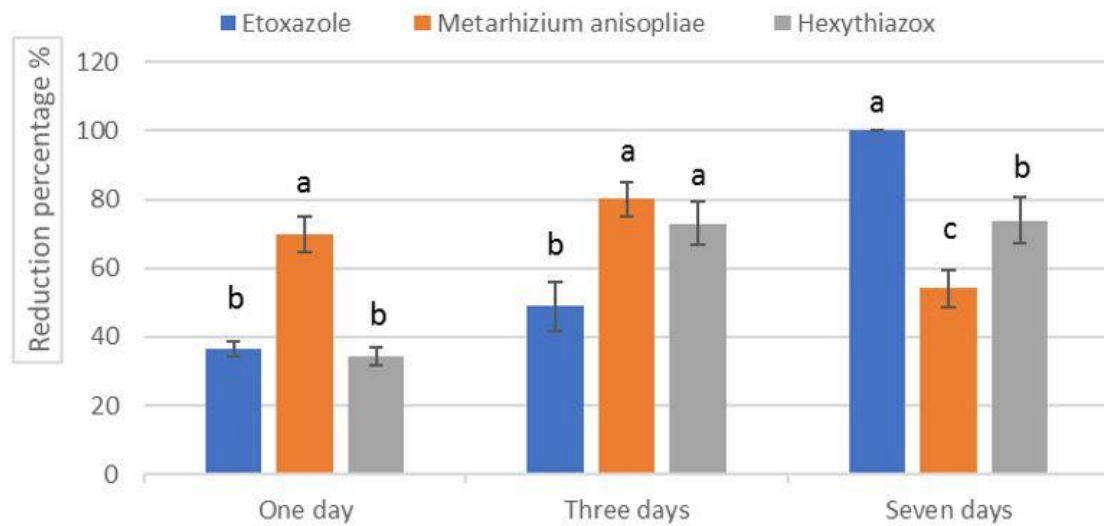


Fig 3. Reduction percentage of *T. urticae* females on pepper plants after 1, 3, and 7 days of application.

The means of the three different pesticides that share the same letters are not significantly different (Duncan's multiple *F*-test at P value ≤ 0.05).

Adulticidal Efficacy on *T. urticae*:

As illustrated in Tables 2 and 3, results showed that the incubation periods for both sexes were prolonged with all treated pesticides compared with control and recorded the longest one in the case of etoxazole (7.2 and 6.8) compared with (5.7 and 5.4) days in control on female and male, respectively, while the immature stages were not significantly affected by treatment. The fecundity of resulting females was significantly reduced in the case of hexythiazox and *M. anisopliae*. At the same time, in etoxazole, it was increased insignificantly compared to the control as the number of eggs per female was recorded at 42.5, 35.3, 77.8, and 77.7 eggs, respectively, with the daily rate of 2.1, 2.75, 3.43 and 3.07 eggs, respectively. There are no significant differences between control and all pesticides applied in egg hatchability% except hexythiazox, which registered 76.33 compared with 99.2 in control. Oviposition periods were significantly reduced in hexythiazox and *M. anisopliae* (19.8 and 13.1 days) and insignificantly reduced in etoxazole (23.1 days)

compared with control (26.2 days). The longevity of females was not significantly different from control in hexythiazox and etoxazole, while in the case of *M. anisopliae*, which lasted significantly shorter when treated with it.

Table 2. Life table parameters (mean \pm S.E in days) of the first generation of *T. urticae* adult females exposed to the tested pesticides in laboratory bioassays.

Biological aspects	Hexythiazox	Etoxazole	<i>Metarhizium anisopliae</i>	Control
Incubation period	6.44 \pm 0.17 b	7.2 \pm 0.13 a	6.0 \pm 0.0 c	5.7 \pm 0.15 c
Immature stages	7.5 \pm 0.48 a	7.3 \pm 0.15 a	7.5 \pm 0.31 a	8.1 \pm 0.35 a
Life cycle	14.0 \pm 0.42 ab	14.5 \pm 0.17 a	13.34 \pm 0.31 b	14 \pm 0.39 ab
Preoviposition period	2.0 \pm 0.26 a	1.2 \pm 13.0 c	1.9 \pm 0.28 b	1.3 \pm 0.15 bc
oviposition period	19.8 \pm 1.8 b	23.1 \pm 1.9 ab	13.1 \pm 0.69 c	26.2 \pm 1.2 a
Postoviposition period	2.1 \pm 0.31 b	4.5 \pm 0.56 a	1.6 \pm 0.31 b	1.7 \pm 0.21 b
Longevity	23.7 \pm 1.9 b	28.8 \pm 2.1 a	16.0 \pm 1.0 c	29.2 \pm 1.3 a
Generation	16.0 \pm 0.44 a	15.5 \pm 0.17 a	15.0 \pm 0.49 a	15.3 \pm 0.39 a
Life span	37.7 \pm 2.11 b	43.3 \pm 2.28 a	30.0 \pm 1.1 c	42.8 \pm 1.47 ab
Fecundity (egg/female)	42.5 \pm 4.76 b	77.8 \pm 6.36 a	35.3 \pm 1.09 b	77.7 \pm 2.72 a
Daily rate	2.1 \pm 0.14 b	3.43 \pm 0.32 a	2.75 \pm 0.18 ab	3.07 \pm 0.18 a
Hatchability	76.33 \pm 6.67 b	96.5 \pm 1.5 a	96.0 \pm 2.1 a	99.2 \pm 0.35 a

Means in a row share the same letters are not significantly different (Duncan's multiple *F*-test at P value \leq 0.05).

For resulted males in Table 3, there are significant differences in the egg incubation period between etoxazole and control, which was the shortest recording (5.4 days) in control compared with (6.0, 6.8, and 6.0) for hexythiazox, etoxazole, and *M. anisopliae* pesticides, respectively. Also, longevity and life span periods were prolonged and significantly different when treated with *M. anisopliae* compared with control and other pesticides.

Table 3. Life table parameters (mean \pm S.E in days) of *T. urticae* adult males resulted from females exposed to the pesticides under investigation (First generation) in laboratory bioassays.

Biological aspects	Hexythiazox	Etoxazole	<i>Metarhizium anisopliae</i>	Control
Incubation period	6.0 \pm 0.32 ab	6.8 \pm 0.2 a	6.0 \pm 0.32 ab	5.4 \pm 0.25 b
Immature stages	7.2 \pm 0.58 a	6.4 \pm 0.25 a	7.4 \pm 0.51 a	6.8 \pm 0.2 a
Life cycle	13.4 \pm 0.68 a	13.2 \pm 0.37 a	13.4 \pm 0.4 a	12.2 \pm 0.2 a
Longevity	5.4 \pm 0.25 b	6.2 \pm 0.37 b	9.0 \pm 0.32 a	6.0 \pm 0.32 b
Life span	18.8 \pm 0.74 b	19.4 \pm 0.24 b	23.4 \pm 0.51 a	18.2 \pm 0.2 b

Means in a row share the same letters are not significantly different (Duncan's multiple *F*-test at P value \leq 0.05)

Ovicidal Efficacy on *T. urticae*:

Two-spotted spider mite eggs were sprayed with the previous three mentioned pesticides. The hatched larvae continued to develop and complete their life cycle, with the exception of etoxazole. It prevented some hatched larvae from reaching the deutonymphal stage. An average total immature duration was recorded at 3.44 days only while their incubation period was (4.78 days). While in the case of hexythiazox, some replicates failed in completing their life cycle, the egg incubation period averaged 4.78 days. Eggs hatched into larvae and completed it with an average of 1.44 days and 77.78% completed the larval quiescent stage (1.29 days). Whilst, 55.56% entered the protonymphal stage (1.6 days) and

33.34% completed the protonymphal quiescent stage (1.67 days). Finally, only 11.11% of individuals became deutonymphs (2 days) and completed the deutonymphal stage (4 days) before death.

As indicated in Table (4), the effect of the tested pesticides on the eggs caused prolongation in the incubation period, immature stages, life cycle, and preoviposition period in the hexythiazox treatment compared with *M. anisopliae* and control. In contrast to oviposition, longevity, and life span periods were significantly decreased recording (13.0, 16.2, and 27.3) compared to (17.8, 21.2 and 31.5) and (20.8, 23.8 and 34.0) for hexythiazox, *M. anisopliae* and control, respectively.

The efficacy of both pesticides was significant on female fecundity as the number of eggs deposited per female, and % hatchability decreased to record (54.5, 43.5 and 68.7 eggs), and (91.47, 92.8 and 99.07%) for hexythiazox, *M. anisopliae* and control, respectively.

Table 4. Ovicidal efficacy of tested pesticides on *T. urticae* females biological aspects in days.

Biological aspects	Hexythiazox	<i>Metarhizium anisopliae</i>	Control
Incubation period	4.0±0.21a	4.0±0.0a	3.5±0.17b
Immature stages	7.1±0.28a	6.1±0.1b	6.7±0.21ab
Life cycle	11.1±0.28a	10.3±0.21b	10.2±0.25b
Preoviposition period	1.6±0.16a	1.3±0.21a	1.4±0.16a
oviposition period	13.0±0.56b	17.8±1.83a	20.8±1.4a
Postoviposition period	1.6±0.16a	2.1±0.43a	1.6±0.22a
Longevity	16.2±0.61b	21.2±2.2a	23.8±1.56a
Generation	12.7±0.26a	11.6±0.26b	11.6±0.22b
Life span	27.3±0.67b	31.5±2.15ab	34.0±1.39a
Fecundity (egg/female)	54.5±2.9b	43.5±2.9c	68.7±1.4a
Daily rate	4.25±0.27a	2.57±0.19b	3.39±0.24b
Hatchability	91.47±0.91b	92.8±3.27b	99.07±0.64a

Means in a row share the same letters are not significantly different (Duncan's multiple *F*-test at P value ≤ 0.05).

While in males resulted from treated eggs with the previous pesticides, as shown in Table 5, there were no significant differences in their incubation periods. At the same time, longevity and life span were significantly decreased with hexythiazox and *M. anisopliae* compared with control recording (3.2, 7.0, and 12.8) and (14.8, 16.1 and 22.2), respectively.

Table 5. Ovicidal efficacy of tested pesticides on *T. urticae* males biological aspects.

biological aspects	Hexythiazox	<i>Metarhizium anisopliae</i>	Control
Incubation period	4.4±0.51a	4.0±0.31a	3.2±0.37a
Immature stages	7.0±0.45a	5.1±0.1b	6.2±0.2a
Life cycle	11.6±0.68a	9.1±0.24b	9.4±0.25b
Longevity	3.2±0.58c	7.0±0.31b	12.8±1.24a
Life span	14.8±0.37b	16.1±0.4b	22.2±1.24a

Means in a row share the same letters are not significantly different (Duncan's multiple *F*-test at P value ≤ 0.05).

Efficacy of the Tested Pesticides on *T. urticae* Egg Hatchability%:

Data in Figure (4) illustrates the hatchability percentage after 24 hours of egg spraying. Etoxazole and hexythiazox have an ovicidal effect compared with *M. anisopliae*. The hatchability percent of the treated eggs with etoxazole, hexythiazox, *M. anisopliae* and

control were 0.6, 9.99, 98.8 and 99.3%, respectively. In the case of treated females, the hatchability percentage of their deposited eggs was 35, 15.69, 97.14 and 100%, respectively (Fig. 5).

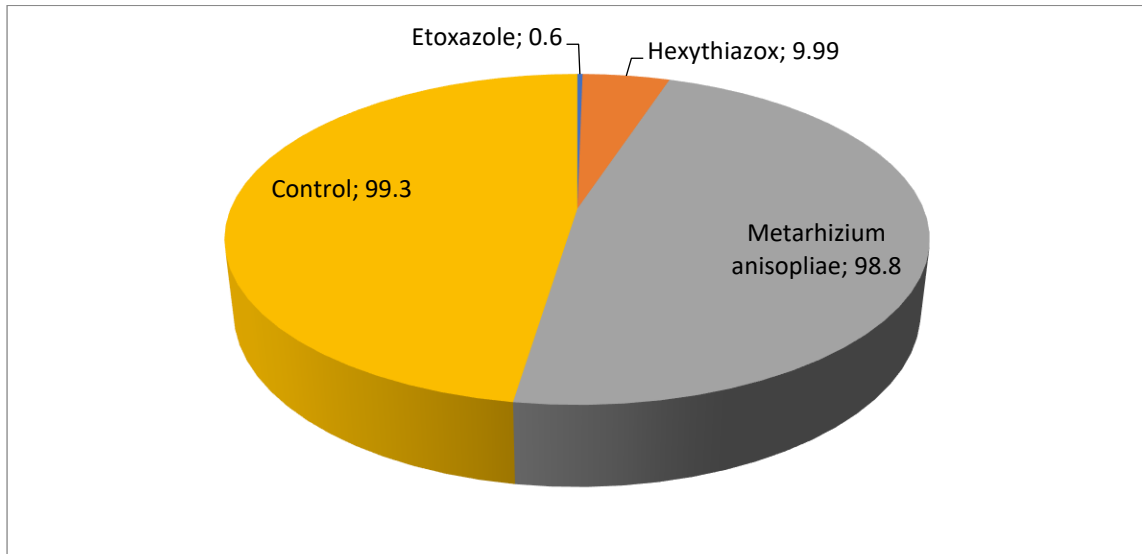


Fig 4. The hatchability percentage of sprayed eggs after 24 hours of spraying with tested pesticides.

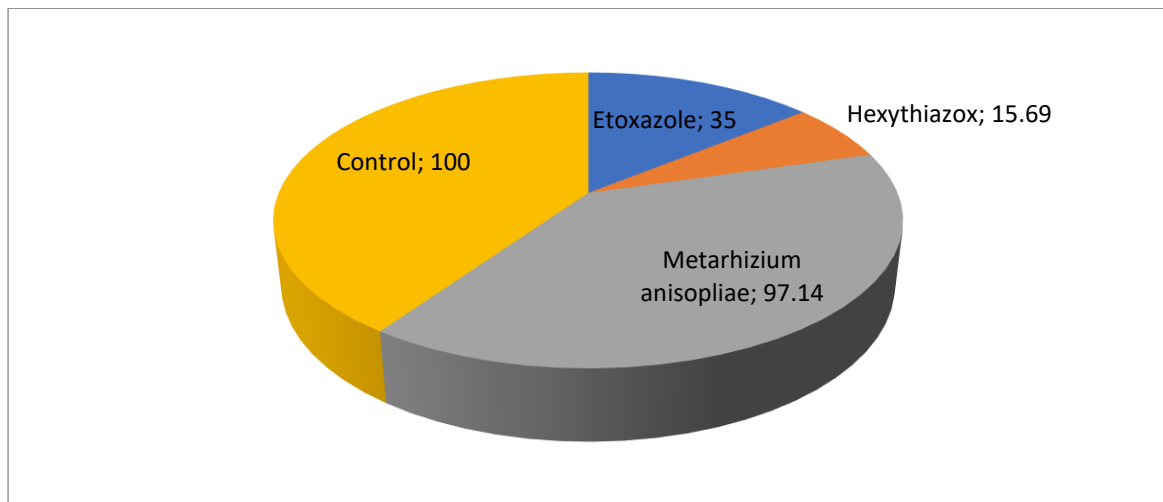


Fig 5. The hatchability percentage of deposited eggs from treated females after 24 hours of spraying with tested pesticides.

DISCUSSION

The two-spotted spider mite became resistant to most traditional pesticides due to repeated application with pesticides. The success of the monitoring method depends on introducing novel modes of action pesticides that help to overcome the resistance phenomena. The efficacy of three formulations of acaricides (hexythiazox, etoxazole, and *M. anisopliae*) against *T. urticae* at different stages was confirmed in this study. The results indicated that *M. anisopliae* biopesticide was the most efficient in reducing *T. urticae* percentages. The current results are comparable to several reports; the *T. urticae* populations in greenhouses were lowered by *M. anisopliae* (Chandler *et al.*, 2005; Bugeme *et al.*, 2015).

A reduction in females of 69.87% was observed after one day of spraying on pepper plants with 250g/100L of *M. anisopliae*. Also, (Keno *et al.*, 2022) showed that *M. anisopliae* strains MF3 and AF5 killed *T. urticae* adults of percentage 64 and 54 % after 8 days of spraying at the rate of 1×10^8 conidia ml⁻¹. The fecundity of females treated with *M. anisopliae* and hexythiazox decreased significantly. However, there was no ovicidal effect of *M. anisopliae* on *T. urticae*. Likewise, (Keno *et al.*, 2022) found that egg mortality was lower than adult mortality when spraying with *M. anisopliae* on tomato leaves. This is because the topography of the eggs is not favorable for the formation of conidia. Additionally, the mite eggshell contains no lipids, crucial nutrients for conidia germination (Keno *et al.*, 2022; Chandler *et al.*, 2005).

Our study also demonstrated the efficacy of etoxazole and hexythiazox as ovicidal, where the egg's hatchability percentages were 0.6 and 9.9 %, respectively. The population of mites is effectively controlled because the treated eggs prevent new generations of pests from emerging. Similarly, etoxazole was more efficient than clofentezine and hexythiazox against eggs of *T. urticae* (Salem and AL-Antary 2012). Additionally, when (López-Manzanares *et al.*, 2022) assessed the effects of etoxazole and spiroadiclofen on larvae and eggs of *T. urticae*, they observed that the eggs had the highest impact. Furthermore, these results revealed that etoxazole and hexythiazox had long-lasting residues and efficiently reduced two-spotted spider mite populations after 7 days of spraying. Biopesticide of *M. anisopliae* showed a low residue level. Consequently, etoxazole and hexythiazole acaricides continue to be effective over an extended period. The ability of these acaricides to persist contributes to reducing the frequency of applications. However, the pests become pesticide-resistant. In order to achieve the best control of two-spotted spider mites, it is essential to alternate these pesticides with *M. anisopliae* biopesticide. However, it avoids the application of etoxazole, followed by hexythiazole. Shumate *et al.*, 2022 reported that etoxazole is capable of building cross-resistance to hexythiazox and clofentazine in *T. urticae* populations.

In this regard, (Reddy *et al.*, 2014) indicated that fenpyroximate and hexythiazox were more effective against two-spotted red mites than dicofol and propargite, and their effects persisted for the 14th day of treatment. Also, the water-dispersible etoxazole formulations-controlled mortality to above 85%. when applied before 14 days of artificially infested with *T. urticae* on the test plants (Cloyd *et al.*, 2009). The results of this study agree with (Saleh *et al.*, 2019) applying fenpyroximate and hexythiazox on eggplant that significantly reduced *T. urticae* populations. In contrast, abamectin 5% did not significantly reduce the population of mites. As well, using hexythiazox resulted in a decrease in the populations of red spider mites compared to the control. Additionally, the phytotoxicity of hexythiazox on plants has not been documented (Shukla, 2018). Moreover, there is no noticeable negative impact of hexythiazox on populations of the predatory mites *Phytoseiulus persimilis* (family: Phytoseiidae) (Sanatgar *et al.*, 2011; Al-Zoubi 2010). While It even improved the fecundity of *T. urticae* females (Nadimi *et al.*, 2008). In another study, the effectiveness of hexythiazox against *T. urticae* came in next to acetamiprid, with reduction percentages in mite population were 21.77, 52.29, 68.94 and 74.56 % after 1, 3, 7 and 14 days of application, respectively. However, hexythiazox exhibited less effect on the predator mite, *Euseius scutalis* (Athias-Henriot) (Family: Phytoseiidae) compared with acetamiprid. After one and three days of spraying, the population decreased by 23.23 and 44.93% for Hexthiazox, while, 39.04 and 65.98% for acetamiprid respectively (Abdel-Rahman 2019). According to (NIU *et al.*, 2014) in two tests evaluating the effectiveness of etoxazole and spiroadiclofen against *T. urticae* on strawberries. In the potting experiment, they noticed that both reduced the immatures by 91% after 3 weeks of treatment. Etoxazole and spiroadiclofen were influential in the greenhouse soil

experiment, decreasing the percentage of adults (87% and 90%) and immature (97% and 81%). In the current study, few hatched larvae resulting from treated eggs by etoxazole were able to enter the deutonymphal stage. Thus, the tested pesticides can effectively manage the immature stages of *T. urticae*.

CONCLUSION

In summary, the biopesticide of *M. anisopliae* and chitin synthesis inhibitors hexythiazox and etoxazole were potent against *T. urticae* through different modes of action and showed potential for pesticide resistance management when rotated. Furthermore, the biopesticide of *M. anisopliae* has a superior effect on reducing mite populations and female fecundity compared to other acaricides. The findings suggested that the tested pesticides are appropriate for the IPM program of mites. Also, they reduce the repetitive application of pesticides because they remain effective for time to seven days of application. Using pesticides that are safe for non-target organisms is essential. In addition, rotational use of them with other pesticides helps manage resistance. Thus, these pesticides require more research in this regard.

Declarations:

Ethical Approval: It is not applicable.

Competing interests: The authors declare no conflict of interest.

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