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Toxicological studies of *Thuja orientalis* plant extract and Vertimec 1.8 EC against two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae) under laboratory conditions

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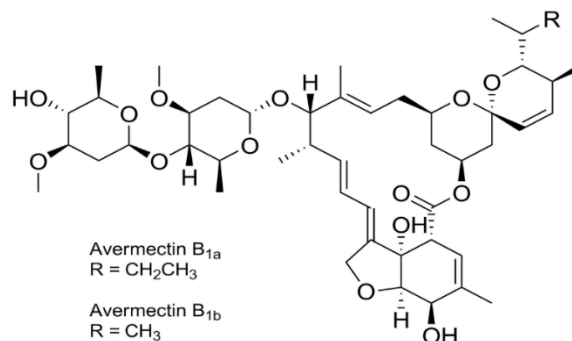
Abstract

Tetranychus urticae Koch is the most harmful pest to many plants in the globe. This previously completed work may offer fresh perspectives on the use of *Thuja orientalis*, a plant extract, as environmentally beneficial acaricide. The effectiveness of various quantities of plant extract from *T. orientalis* against adult females of the two-spotted spider mite *T. urticae*, with LC₅₀ values of 8.35, 6.310, and 4.48 after one day, two days, and three days, respectively. In addition to the abamectin (vertimec 1.8 EC) acaricide's acaricidal efficacy at various concentrations, where the LC₅₀ values were 1.02, 2.74, and 1.65 after one day, two days, and three days, respectively. Furthermore, the adult fecundity, repellency, and hatchability of *T. orientalis* plant extract and vertimec varied substantially with concentration. The findings of this study suggested that the plant extract of *T. orientalis* might be used as a bio acaricide compared with vertimec to control spider mites.

Keywords: Plant extract, Acaricidal activity, *Thuja orientalis*, *Tetranychus urticae*, Acari, phytophagous mite, Vertimec, Toxicity, and Repellency.

INTRODUCTION

One of the most pervasive plant pests in the world is the *Tetranychus urticae* Koch (Acari: Tetranychidae), which affects horticultural, ornamental, and agricultural crops everywhere (James and Price, 2002; Adesanya et al., 2021 and Shukla, 2021) and In Egypt, *T. urticae* Koch is a well-known pest that causes considerable yield losses in various economic crops, vegetables, and fruit trees (Salman, 2007). Its feeding behavior, which occurs on the underside of leaves, leads to the removal of essential chlorophyll, causing a decrease in photosynthetic activity. Due to their frequent application of acaricides, the pest's rapid reproduction and brief life cycle may make it easier to build resistance. (Stumpf and Nauen, 2002; Steinkraus et al., 2003; Chiasson et al., 2004 and Van Leeuwen et al., 2005 and Taha et al. 1990). Spider mite control has predominantly depended on the application of acaricides, causing the development of pesticide resistance and the accumulation of chemical residues on the collected produce (Attia et al. 2013). Essential oil, mineral, and plant extract tend to be less harmful to predatory mites compared to certain widely used prepared miticides, which are typically safer for both the environment and human health in contrast to prepared miticides. (Momen and Amer, 2003; Momen et al. 2001). Up until now, numerous studies have explored the application of essential oils and various plant extracts for the management of phytophagous mites (Momen et al. 2001; Choi et al. 2004; Miresmailli and Isman 2006; Han et al. 2010 and Hussein et al. 2013). A common anthelmintic and acaricide is abamectin, it is a naturally occurring fermentation byproduct of the actinomycete *Streptomyces avermitilis* that lives in soil, and it belongs to the avermectin family (Ikeda H. et al. 2003). A double bond on C-22-25 distinguishes abamectin from ivermectin, the most well-known member of the avermectin family. When *Streptomyces avermitilis* is fermented, 8 closely similar avermectin homologues are produced, with B1 constituting the majority of the fermentation. B1a (about 80%) and B1b (about 20%) are combined to form the nonproprietary name abamectin. Abamectin is the only Avermectin that has applications in both agriculture and medicine (Jansson R. K. et al. 1998).



Chemical structure of avermectin B1a and B1b.

The nerve and muscle cells of invertebrate organisms contain glutamate-gated chloride channels, which avermectins bind to. These cells become hyperpolarized as a result, paralyzing them and eventually killing them. Because they have little affinity for other mammalian ligand-gated channels and seldom cross the blood-brain barrier, avermectins are extremely safe for mammals since they are only found in the brain and spinal cord of mammals (Shoop W. L. *et al.* 1995). In order to investigate the possibility of using *T. urticae* plant extracts and vertimec 1.8 EC) as an alternative to acaricidal, as well as the toxicity of *T. orientalis* plant extracts against adult females, adult fecundity, repellency, and hatchability, this experiment was carried out.

MATERIALS AND METHODS

1. Plant material:

1. A) *Thuja orientalis* Plant extract:

The plant extract was extracted from the leaves of *T. orientalis*, they were gathered from the Agricultural Research Centre, Shandaweel Research Station, Sohag governorate, Egypt, and the Plant Protection Research Institute. The choice of plant species was informed by earlier studies and the utilization of plant-derived products in indigenous medicinal practices.

1. B) Chemicals:

Vertimec: Abamectin (1.8 % E.C)

2. Preparation for extraction:

For almost 15 days, *T. orientalis* air-dried the plant components she had collected. They were sieved with a 40-mesh sieve to achieve a constant particle

size after drying, then processed using a grinding mixer (Thomas-Wiley Laboratory mill, Model 4). The powdered substance was then kept at room temperature in polythene bags until extraction. The extraction procedure followed the established guidelines provided by (Cseke et al. 2006) and used an organic solvent and a soxhlet extractor (Hot Continuous Extraction). A thimble containing 20 g of powder was successively extracted with 70% ethanol for around 48 hours, or until the eluting solvent in the thimble turned colorless. After employing a rotary evaporator to remove the solvent, the crude extracts were weighed and kept in a refrigerator. The required number of extracts were gathered by repeating this technique several times.

3. Rearing of *T. urticae*.

The Shandaweel Research Station farm supplied the original colony of the spider mite *T. urticae* Koch with severely afflicted fig orchard leaves in the 2022 growing season. Castor leaf discs were placed in foam dishes (15x20 cm) in the lab with the lower surface facing up and on wet cotton pads supported by sponges. The colonies were kept alive in a lab environment at ambient temperature. The edges of the leaf discs had damp, fine cotton barriers all around them to keep mites from escaping. Every three days, leaf discs were examined, and if evidence of mite overcrowding or leaf yellowing was found, fresh leaf discs were substituted. The environment in which each bioassay was carried out and administered was the same as that of the culture.

4. Laboratory Bioassay.

4.1. Preparation of the test concentrations from vertimec 1.8 EC and *T. orientalis* extract:

In our study, the toxic effect of abamectin an Acaricide (Vertimec 1.8 EC) and plant extract (*T. orientalis*) against the adult of *T. urticae* has been evaluated. Thus, five concentrations of vertimec (reference acaricide 1.8 EC 0.18, 0.37, 0.75, 1.5, and 3 ppm) has been used in toxicological bioactivity. To achieve the target concentrations of 0.125, 0.25, 0.5, 1.0, and 1.5 ppm, a known quantity of *T. orientalis* crude extract was dissolved in the appropriate solvent in a 1:1 proportion and serially diluted with distilled water. 0.1% tween-80 was used as a surfactant, and the

concentrations were chosen based on prior studies. The acaricidal activities of *T. orientalis* plant extract and abamectin an acaricide (Vertimec 1.8 EC) were measured by using the leaf dipping method under the same reported laboratory conditions (Kabir et al. 1993 and Ladhari et al., 2013). With a new binocular microscope the *T. urticae* mortality was taken after three days of treatment. *T. urticae* that were unable to move was considered dead.

4.2. Treatment adult females of *T. urticae*:

The 5 concentrations of *Thuja orientalis* and vertimec 1.8 EC were applied for approximately 10 seconds to thirty replicate castor leaf discs (2 cm in diameter) (Ladhari et al., 2013). Leaf discs used as controls were submerged in distilled water with 0.1% tween-80 and allowed to air dry. Using a fine camel toothbrush, ten adult females of *T. urticae* were placed on the lower surface of each disc of castor leaf disc (10 adult females/disc). One of the earlier treatments was applied to leaf discs. Three copies of each treatment were performed. Under a stereomicroscope, mortality was noted 24, 48, and 72 hours after treatments. When poked with a delicate camel toothbrush, mites were thought to be dead if their bodies or appendages did not move (Kim et al. 2004).

4.3. Effect of plant extract on fecundity and mortality of *T. urticae* females:

Leaf discs from castor plants were dipped in various concentrations of *Thuja orientalis* plant extracts, vertimec 1.8 EC, and 0.1% tween-80. The results showed that the plant extract decreased the fertility and death of *T. urticae* females. Females that had just emerged were moved on separate leaf discs. For each concentration, three replicate leaf discs were employed, and a similar number of females on clean leaf discs served as the control. For seven days, female fecundity and death rates were tracked. According to Lundgren (1975), the oviposition deterrent indices (ODI) were calculated

4.4. Repellency Bioassay

Castor bean leaf discs (5 cm in diameter) were made by arranging the discs with their surfaces turned upside-down in Petri dishes. Five concentrations of 0.18, 0.37, 0.75, 1.5, and 3 ppm for vertimec 1.8 EC and 0.125, 0.25, 0.5, 1.0, and

1.5 ppm for *T. orientalis* plant extracts with 0.1% tween-80 for two compounds were applied to half of each disc for ten seconds then allowed to dry. The other half was left untreated as a check. Using a fine camel hairbrush, ten females of the same age were positioned in the center of the leaf disc. The number of mites on the treated and untreated half was counted after 12, 24, and 48 hr. For each oil concentration, 3 replications of leaf discs were used; each treatment was repeated three times. The repellency index was calculated according to (Pascual-Villalobos and Robledo 1999) as follows: $RI = (C-T / C+T) \times 100$, where: the number of treated mites and C: the number of untreated mites.

4.5. Toxicity of tested *Thuja orientalis* plant extracts and vertimec 1.8 EC to hatchability of eggs of two-Spotted Mite *T. urticae*:

Ten adult females were placed on cotton leaf discs (2 cm in diameter) on wet cotton wool in a petri dish and allowed to lay eggs. The petri dish was then incubated for 24 hours under laboratory conditions to examine the effects of the tested *T. orientalis* plant extracts and vertimec 1.8 EC). The female adults were then taken out of the leaf discs. Eggs were sprayed by a glass atomizer in each concentration of *T. orientalis* plant extracts and vertimec 1.8 EC concentrations and allowed to dry. Each concentration was prepared in four replicates, with each replicate containing three cotton leaf discs and ten eggs. Water and 0.1% tween-80 were sprayed upon untreated discs. The test eggs were kept at room temperature in a controlled environment for 10 days prior to hatching. Egg-hatching and non-hatching rates were counted. Egg-hatching and non-hatching rates were counted. 1983's (Craveiro et al.)

Statistical analysis

The corrected mortality rate for each of the previously synthesized compounds is statistically calculated according to (Finney 1971). The average mortality percentage was corrected using Abbott's formula (Abbott 1925). The toxicity index of tested compounds was determined according to (Sun 1950).

RESULT AND DISCUSSION

1) Treatment of adult females of *T. urticae*:

A) Toxicological activity of abamectin insecticide (vertimec 1.8 EC) towards adult of two-spotted spider mite *T. urticae* after 1-3 days of treatment.

Data in Figure 1 & Table, 1 revealed result that all tested vertimec Acaricide exhibits moderate to weak toxicological activity against the *T. urticae* with LC_{50} values varied from 1.029 to 2.747 ppm after 3 days of treatment. In particular LC_{50} values of vertimec after one day, two days, and three days are 1.029, 2.747, and 1.657 ppm respectively. These results agree with (Ismael 2007) who reported that the study investigated the mortality rates of adult female subjects treated with different concentrations of abamectin. its results suggest that higher concentrations of abamectin were associated with increased mortality rates, with the highest concentration of 2.5 ppm resulting in a mortality rate of $96.3 \pm 0.33\%$ after 24 h. (Chiasson et al. 2004) stated that abamectin was identified as the most potent unconventional compound, exhibiting a mortality rate of 94.9% against the adult stage of TSSM.

Table 1: The acaricidal efficacy of different concentrations of abamectin (vertimec 1.8 EC) as reference acaricide and *Thuja orientalis* plant extract after one, two, and three days of treatment.

Vertimec 1.8% EC					<i>T. orientalis</i> extract			
Days	LC_{50}	Slope	X^2	Toxicity index ^a	LC_{50}	Slope	X^2	Toxicity index
1	1.029	0.976 ± 0.251	16.76	1	8.354	893.0 ± 0.335	3.017	0.537
2	2.747	0.453 ± 0.243	1.123	0.374	6.310	0.428 ± 0.344	0.647	0.711
3	1.657	0.596 ± 0.244	0.760	0.621	4.488	0.429 ± 0.234	3.517	1

Notes: Toxicity ratio^a is calculated as the least LC_{50} value for baseline toxicity / the compounds' LC_{50} value.

B) Toxicological activity of *Thuja orientalis* extract towards adult of two-spotted spider mite *T. urticae* after 1-3 days of treatment.

From data in Figure 1 and Table 1, the revealed results that all tested *Thuja orientalis* gave LC₅₀ values varied from 4.488 to 8.354 ppm after 3 days of treatment. In particular LC₅₀ values of *Thuja orientalis* after one day which is represented by a bold line, two days represented by a dashed dot line, and after three days of treatment which is represented by a dotted line, are 8.354, 6.310, and 4.488 ppm, respectively. From this result, the LC₅₀ value of Vertimec after one day of treatment was the most affected than the other values. Furthermore, LC₅₀ value of *T.*

orientalis extract after one day of treatment was the most affected than the other values. These results agree (El-Gepaly, et al., 2016) who reported that the extracts also achieved high mortality rates. The mortality rates during the pre-oviposition and oviposition stages were 65.52% and 51.72%, respectively, at the maximum concentration of Artemisia extract. The LC₅₀ values for the adult females of *T. urticae* after 72 hours were 1.28, 0.85, 0.53, 1.61, 0.44, 3.11, and 0.46%, respectively (Elhalawany and Dewidar, 2017). Seven plant essential oils, including lemon grass, spearmint, rosemary, marjoram herbs, fennel and coriander seeds, and chamomile flowers, were tested for their toxicity against adults of *T. urticae* Koch.

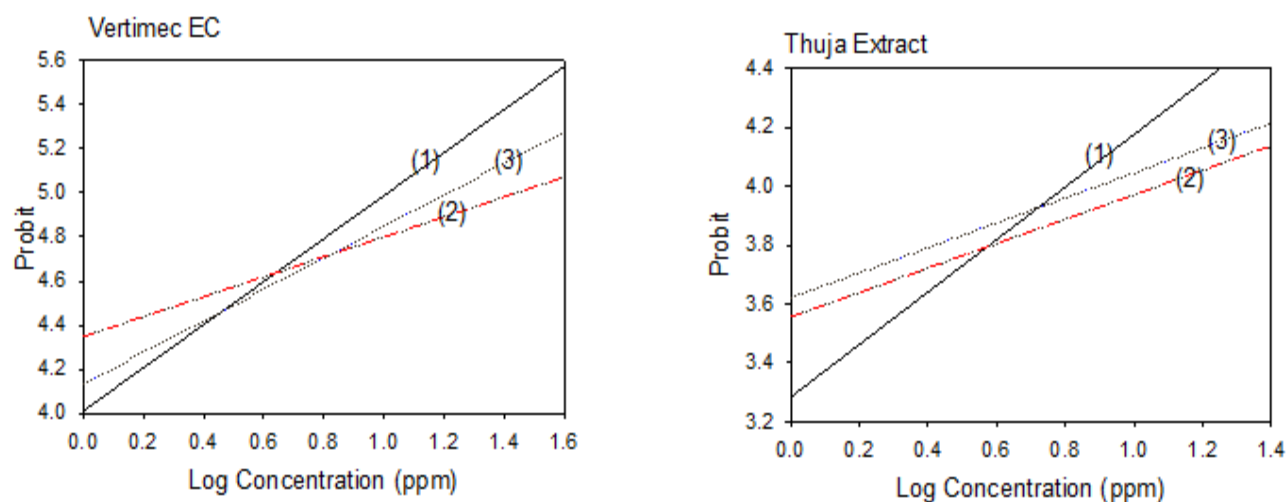


Figure 1: The acaricidal efficacy for abamectin acaricide (vertimec 1.8 EC) and recently different concentrations of *Thuja orientalis* plant extract against the adult females of two-spotted spider mite *T. urticae* in which after one day of treatment represented by Bold line, after two days of treatment represented Dash Dot line and after three days of treatment represented by Dot line.

2. Effect of plant extract on fecundity of *T. urticae* females:

Table 2: The percentage of the number of eggs laid by adult females of the two-spotted spider mite *T. urticae* after exposure to the *Thuja orientalis* extract and vertimec acaricide after one, two, and three days of treatment.

<i>T. orientalis</i> extract				Vertimec acaricide			
Conc.	Day 1	Day 2	Day 3	Conc.	Day 1	Day 2	Day 3
0.125	30%	26.60%	16%	0.18	26.60%	10%	0%
0.25	20.60%	16.60%	16.60%	0.37	3.30%	0%	3.30%
0.5	10%	10%	6.60%	0.75	6.60%	6.60%	3.30%
1	6.60%	6.60%	3.30%	1.5	6.60%	0%	0%
1.5	3.30%	3.30%	2.60%	3	3.30%	0%	0%

As shown in Table 2 the percentage of the eggs laid by adults of *T. urticae* which treatment with the *T. orientalis* extract and vertimec acaricide, after day one of the treatments with *T. orientalis* extract the highest concentration gave 3.30 %, after two days gave 26.60 % and after three days of treatment gave 16.16 %. However, under 1.50, 1, 0.5, 0.25, and 0.125 ppm dose of concentrations and after day 2 days gave 3.30, 6.60, 10, 16.60 ad 26.60 %, respectively. In addition, after three days of treatment gave 2.60, 3.30, 6.60, 16.60 ad 16%, respectively. As shown in Table 2 the percentage of the eggs laid by an adult of *T. urticae* which

treatment with vertimec as a reference acaricide, after day one the highest concentration of 3 ppm gave 3.30 %, after two days gave the 0 %, and after three days of treatment gave 0 %. Which under 1.5, 0.75, 0.37, and 0.18 ppm doses after two days of treatment gave 0, 6.60, 0, 10 %, which after three days of treatment gave 0, 3.30, 3.30, and 0 %. (Ismael, 2007) who studied that the abamectin was more potent in its effect on fecundity (2.5 ± 0.39 eggs/female) compared to the untreated-control group (6.67 ± 0.5 eggs/female). Chiasson et al. (2004) who reported that abamectin reduced the fertility of TSSM adult females.

3. Repellency Bioassay

Table 3: The repellent effect of the *Thuja orientalis* extract and vertimec as a reference acaricide against adult females of the two spotted spider mite *T. urticae*.

<i>T. orientalis</i> extract			Vertimec acaricide		
Conc.	Treated	Untreated	Conc.	Treated	Untreated
0.125	6.60%	86.60%	0.18	23.30%	73.30%
0.25	6.60%	83.30%	0.37	26.60%	63.30
0.5	16.60%	76.60%	0.75	36.60%	66.60%
1	20%	73.30%	1.5	43.30%	56.60%
1.5	36.60%	63.30%	3	66.60%	33.30% %

Repellency effect of plant extract, as shown in Table 3 the repellency effect of *T. orientalis* plant extract and vertimec as a reference acaricide on adults of the two-spotted spider mite. Plant extract and vertimec had a significant repellency on adults of *T. urticae*. The Efficacy of the high dose (1.5 ppm) of *T. orientalis* was more than others which gave 36.60 % for treated and 63.30 % for untreated. However, the efficacy of the dose 1, 0.5, 0.25, and 0.125 ppm gave 20, 16.60, 6.60, and 6.60, respectively for treated and 73.30, 76.60, 83.30, and 86.60, respectively for untreated. The essential oil of *T. orientalis* has repellent action and reduces the fecundity of *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae) (Papachristos and Stamopoulos, 2002). Hanifah et al. (2012) found that the plant extracts exhibited varying degrees of repellency against larval *Leptotrombidium deliense* (Acari: Trombiculidae), and the level of repellency increased with higher concentrations of the extracts. An onion extract was found to have the highest repellency at the lowest concentration of 0.01%, with a rate of 30%, followed by clove and cinnamon extracts at 23%.

Araújo et al. (2012) reported that the eugenol component had a strong repellency property on *T. urticae*.

In addition, the efficacy of the high dose of vertimec acaricide (3 ppm) was more than others which gave 66.60 % for treated and 33.30 % for untreated. However, the efficacy of the doses 1.5, 0.75, 0.37, and 0.18 ppm gave 43.30, 36.60.30, 26.60, and 23.30, respectively for treated and 56.60, 66.60, 63.30, and 73.30, respectively for untreated

4. Toxicity of tested *T. orientalis* plant extracts and vertimec 1.8 EC to hatchability of eggs of two-Spotted Mite *T. urticae*:

As shown in Table 4 after ten days of treatment, the mean number of eggs laid by adults was significantly different between other concentrations, which under-treated by the height's concentration 1.5 ppm after eight days of treatment of *T. orientalis* extract gave 16.60 %, after nine days of treatment gave 33.30% and after ten days of treatment gave 0%. However, under 1, 0.5, 0.25,

and 0.125 ppm dose and after eight days gave 13.30, 13.30, 33.30, and 30.30, respectively. After nine days of treatment gave 26.60, 56.60, 53.30, and 53.30, respectively. These results agree (El-Gepaly, et al., 2016) who stated that the extracts also led to a reduction in hatchability, with *Artemisia* 0.25% and 0.5% and *Acacia* 0.25% and 0.5% achieving hatchability reductions of 61.11%, 57.11%, 54.11%, and 52.11%, respectively. These findings suggest that the extracts have the potential as effective natural alternatives to conventional acaricides for controlling mite populations.

In addition, after ten days of treatment gave 20, 3.30, 10 and 13.30 %. As shown in Table 4 after eight days of treatment by vertimec as reference acaricide, the mean number of eggs laid by adults was significantly different between other concentrations, which under-treated by the height's

Table 4: The effect of the *Thuja orientalis* extract and vertimec as a reference acaricide on hatching eggs after eight, nine, and ten days of treatment.

<i>T. orientalis</i> extract				Vertimec acaricide			
Conc.	Day 8	Day 9	Day 10	Conc.	Day 8	Day 9	Day 10
0.125	30.30%	53.30%	13.30%	0.18	66.60%	3.30%	6.60%
0.25	33.30%	53.30%	10%	0.37	66.60%	23.30%	6.60%
0.5	13.30%	56.60%	3.30%	0.75	46.60%	33.30%	0%
1	13.30%	26.60%	20%	1.5	43.30%	23.30%	0%
1.5	16.60%	33.30%	0%	3	40%	10%	0%

CONCLUSION

In this work, it was thought to be worthwhile to build up *Thuja orientalis* plant extract that act as an ecofriendly acaricide. New insights into the application of plant extract of the oriental arbor-vitae, *T. orientalis* as novel bio-acaricides. The acaricidal efficacy of different concentrations of *T. orientalis* plant extract after 3 days against adult females of two-spotted spider mite *T. urticae* in which LC₅₀ was 8.35, 6.310 and 4.48 after one day, two, and three days, respectively. In addition to the acaricidal efficacy of different concentrations of abamectin (vertimec 1.8 EC) as reference acaricide in which LC₅₀ was 1.02, 2.74, and 1.65 after one day, two, and three days, respectively. Furthermore, the adult repellency and oviposition deterrent of *T. orientalis* plant extract varied substantially with

concentration (3 ppm) gave 0 % after seven days of treatment, gave 40 % after eight days of treatment, 10 % after nine days of treatment and after ten days of treatment gave 0 %. However, 1.5, 0.75, 0.37 and 0.18 ppm of concentration gave 0, 3.30, 0, and 3.30 %, respectively after seven days of treatment. Which are 43.30, 46.60, 66.60, and 66.60 %, respectively after eight days of treatment. After nine days of treatment gave 23.30, 33.30, 23.30, and 3.30 %. Finally, after ten days of treatment gave 0, 0, 6.60, and 6.60 %. These result harmony Baranowski (1996) findings, the emergence of *T. urticae* Koch eggs that were treated with low concentrations of abamectin did not result in any toxicity. Salman (2007) reported that abamectin exhibited high toxicity toward eggs of all ages. Chiasson et al. (2004) report, abamectin decreased the fertility of adult female TSSM.

concentration. This study's results encourage further testing of these promising compounds, & the Exploitation of their toxicological activity to produce new eco-friendly acaricides against two-spotted spider mite *T. urticae*. Overall, the conclusion suggests that abamectin at higher concentrations is associated with increased mortality rates in adult female subjects.

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