



# Innovative Approach for the Use of Huwa-San TR50 in Controlling Two Spotted Spider Mite *Tetranychus urticae* Koch (Acari: Tetranychidae)

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## ABSTRACT

The efficacy of Huwa-San TR50 against two spotted mite, *Tetranychus urticae*, on tomato plants, was tested under greenhouse and laboratory conditions. A sharp reduction in the population of *T. urticae* was obtained after one week of applying Huwa-San TR50 at a rate of 4000 ppm. This resulted in mortalities of 82.10 and 78.60% under greenhouse and laboratory conditions, respectively. The side effects of Huwa-San TR50 on predatory mite, *Neosius cucumeris*, were also tested to gain successful implementation of new integrated pest management. After the direct application of Huwa-San TR50, *N. cucumeris* could also sustain its population on treated and/or dead *T. urticae*, under laboratory and greenhouse conditions. Statistically, the difference between laboratory and greenhouse conditions was insignificant ( $P > 0.5$ , using F-test Graphpad prism 7). Up to 3000 ppm of Huwa-San TR50 reduced the population of *N. cucumeris* by 11.83 and 17.66% under greenhouse and laboratory conditions, respectively. However, 4000 ppm of Huwa-San TR50 reduced the *N. cucumeris* population by ~3-fold of the reduction gained with 3000 ppm in both treatments. Moreover, the maximum mortality was held between 3000 and 4000 ppm with no significant difference in the mortality of *T. urticae*. Finally, Huwa-San TR50 can be safely used in biological control programs, as it has little or no effects on biological control agents such as predatory mite, *N. cucumeris*, which is widely associated with the infestation of two spotted spider mite, *T. urticae* on tomato plants.

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SSA and MMA equally contributed in designing experiment, analysing the collected data and writing up the manuscript.

## Key words

Huwa-San TR50, *Tetranychus urticae*, *Neosius cucumeris*.

## INTRODUCTION

The two spotted spider mite (TSMM), *Tetranychus urticae* Koch is considered as the most important species of the family Tetranychidae, as it is associated with 900 plant species (Jeppson *et al.*, 1997; Meyer, 1996). It attacks ornamental plants, orchard trees and medical plants (Naher, 2005). It has been reported that *T. urticae* can significantly cause economic loss in cotton, beans, tomatoes, peppers, strawberries and roses (Niu *et al.*, 2014; Kumari *et al.*, 2015). Like other phytophagous mites, *T. urticae* feeds on plant leaves and produces injuries to the epidermis which result in yellow and brown blotch that is accompanied by dryness and leaf fall (Abou-El-Ela, 2014). In addition to its damage to the plant, *T. urticae* infestation can cause a marked reduction in the quantity and quality of several economic crops or in complete yield loss (Niu *et al.*, 2014).

The toxicity of novel and conventional acaricides such as spiromesifen, hexythiazox, abamectin, chlorfenapyr,

fenpyroximate, matrine, beta-cypermethrin, etoxazole, bifenazate, spiroadiclofen, pyridaben, diafenthiuron, fenazaquin, dicofol, clofentezine and dimethoate to all *T. urticae* stages was evaluated in several recent studies (Niu *et al.*, 2014; Shah and Shukla, 2014; Kumari *et al.*, 2015). These include plant extracts especially Neem (*Azadirachta indica* A. Juss), which is based on the commercial formula, azadirachtin (Bernardi *et al.*, 2012). Furthermore, the side effects of these acaricides to predatory mites associated with the infestation of *T. urticae* were reported in these studies. This is because the use of pesticides is usually necessary to maintain the pest population below the economically acceptable level which cannot be gained by predation (Maroufpoor *et al.*, 2016). Therefore, many acaricides have been extensively used for controlling *T. urticae*, thereby resulting in a rapid resistance to many acaricides including abamectin, dicofol, clofentezine and hexythiazox, after a few years of introduction (Beers *et al.*, 1998; Stumpf and Nauen, 2001, 2002; Sato *et al.*, 2005; Whalon *et al.*, 2008; Nicastro *et al.*, 2013; Tirello *et al.*, 2012). Therefore, growers demand for acaricides that have long residual toxicity to suppress the high-density of *T. urticae* but the use of these acaricides might promote

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resistance (Shah and Shukla, 2014).

On the other hand, controlling *T. urticae* might affect not only the target species but the non-target species as well, such as the beneficial and predatory insects/mites which are associated with the infestation of other pests. For example, it has been recorded that abamectin is toxic to predatory mites, *N. californicus* (McGregor, 1954) (Acari: Phytoseiidae) and *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae) which are the main predators associated with *T. urticae* in the strawberry crops of Brazil (Bernardi *et al.*, 2012). Furthermore, abamectin has been reported to be toxic to all stages of Asian ladybird beetle, *Harmonia axyridis*, except eggs (Youn *et al.*, 2003) that feed on several aphid species (Seo and Youn, 2000). In addition, the use of pesticides was also found to impair the performance of natural enemies which are more susceptible to pesticides than the phytophagous mites (Croft, 1990). However, the main problem is the high amount of acaricide left over, as harvesting of several crops and/or fruits are performed daily. Therefore, this has encouraged several researchers to develop an alternative management strategy for the control of *T. urticae* with minimal side effects on non-target species. Alhewairini (2017) was the first to successfully use Huwa-San TR50 in controlling cotton aphids (*Aphis gossypii* Glover) without significant effects on honeybees (*Apis mellifera lamarckii*) and seven-spot ladybird beetles (*Coccinella septempunctata*).

According to the literature review, Huwa-San TR50 has not been used for the control of *T. urticae* and its side effects on the predatory mite *N. cucumeris* are still unknown. Huwa-San TR50 was developed over twenty years ago and is widely used as a disinfectant ([www.huwasan.com](http://www.huwasan.com)). Huwa-San TR50 is a formulation of hydrogen peroxide which has been stabilized by the addition of a small quantity of silver ([www.huwasan.com](http://www.huwasan.com)). There are several advantages that make it reliable and safe, such as its high efficacy even at low concentrations, being effective under a wide range of temperatures up to boiling point, being gentle to the skin, having long term effectiveness, being biodegradable, having no build-up of resistance by microorganisms, as well as being non-toxic to humans, colorless, tasteless and odorless ([www.huwasan.com](http://www.huwasan.com)).

The present study aimed to test the efficacy of Huwa-San TR50 against two spotted spider mite (*T. urticae* Koch) and its side effects on the associated predatory mite, *N. cucumeris* Oudemans.

## MATERIALS AND METHODS

Huwa-San TR50 was obtained from Ghatafan Company in Onaizah (retailer agent). The field experiment

was carried out throughout the January 2017 season in an abandoned tomato (*Solanum lycopersicum* L.) greenhouse, having a history of spider mite infestations. The Experimental Research Station, Qassim University, Buraidah, Al-Qassim, Saudi Arabia was selected for studying the effect of chemical control on this phytophagous mite species using four different concentrations of Huwa-San TR50: 1000, 2000, 3000 and 4000 ppm (already used by Alhewairini, 2017) including the control (well water). The stock solution of Huwa-San TR50 (500,000 ppm) was diluted with well water to give a serial concentration range between 1000 to 4000 ppm.

The chosen area was about 10 m<sup>2</sup> and was already cultivated with tomato seedling (Pritchard cultivar). The whole area was divided into 5 plots (each plot was about 2 m<sup>2</sup>), and all plots were arranged in a randomized complete block design.

Twenty leaves of each treatment were randomly collected and placed directly into plastic bags separately and transported to the laboratory. All mite stages were counted using a stereomicroscope, to determine the initial distribution and density of the mites as pre-spray counts. Observations were made after 1, 2, 3 days and one week after the application of four Huwa-San TR50 concentrations by using a conventional hand sprayer to evaluate the reduction percentage of the *T. urticae* populations on the tomato plants. In greenhouse experiments, four different Huwa-San TR50 concentrations (1000, 2000, 3000 and 4000 ppm) including the control (well water) were sprayed directly on the tomato plants (mid-stage) by using a knapsack sprayer (20 L).

The control of *T. urticae* by Huwa-San TR50 on potted bean plants was also conducted in the Laboratory, Department of Plant Production and Protection, College of Agriculture and Veterinary Medicine, Qassim University. *T. urticae* and *N. cucumeris*, in moving stages, used in this study were collected from heavily infested tomato leaves at an abandoned greenhouse and transferred to rearing substrates consisting of bean plants in the laboratory. Bean leaves (*Phaseolus vulgaris* L.), 4.0 cm in diameter, were used as rearing arenas in plastic Petri-dishes (5 × 1 cm) with lower surface facing upwards. The cotton bed was kept wet by soaking with distilled water twice daily to keep leaf discs fresh and to avoid the migration of mites to the lower leaf surface. With the aid of a binocular microscope and with a fine paint brush, 30 moving stages of *T. urticae* were transferred to the Petri dish, and 10 predatory mites, *N. cucumeris*, were transferred to another Petri dish for measurement of its vulnerability to Huwa-San TR50. Before transferring mites to the leaf discs, the leaf discs were checked under a microscope to avoid the presence of predatory insects and mites. To prevent mites from

escaping, a ring of Vaseline was put around the leaves. Four different concentrations of Huwa-San TR50 (1000, 2000, 3000 and 4000 ppm) as treatments (already used by Alhewairini, 2017) and one untreated group as a control (well water), were sprayed directly on the Petri-dishes by using a small knapsack sprayer (1 L).

#### Statistical analysis

The reduction percentages of the average population number of phytophagous and predatory mite species were calculated according to the equation of Henderson and Tilton (1955):

$$\text{Corrected (\%)} = \left(1 - \frac{n \text{ in C before Tr} \times n \text{ in T after Tr}}{n \text{ in C after Tr} \times n \text{ in T before Tr}}\right) \times 100$$

Where,  $n$  is number of *T. urticae* or *N. cucumeris* population,  $T$  is treated,  $C$  is control and  $Tr$  is treatment.

The mortalities of the two spotted spider mite (*T. urticae* Koch) and the predatory mite (*N. cucumeris* Oudemans), were calculated manually by direct observation. Thereafter, the obtained data were calculated by using Microsoft Excel. The obtained data for all variables were

statistically analyzed using One-way analysis of variance (ANOVA). Curves for the mortality assays were plotted using Graphpad Prism version 7. The data points were the mean  $\pm$  SEM of each treatment with Huwa-San TR50 and the graphs were fitted using a non-linear regression (log (inhibitor) vs. normalized response- variable slope) with a four parameter logistic equation where the upper plateau was set to 100% and the lower plateau was set to 0. The results are expressed as mean mortality percentage  $\pm$  SEM for each treatment.

## RESULTS

The potential toxic effects of Huwa-San TR50 on two spotted spider mite (*T. urticae* Koch) and the associated predatory mite (*N. cucumeris* Oudemans) on tomato plants was tested. Under greenhouse conditions, the mortality percentage was 27.70, 59.10, 81.73 and 82.10% for *T. urticae* and 11.41, 16.73, 17.66 and 42.11 % for *N. cucumeris* at 1000, 2000, 3000 and 4000 ppm of Huwa-San TR50 one week after treatment, respectively (Tables I and II).

**Table I.- Effect of four concentrations of Huwa-San TR50 on two spotted spider mite (*Tetranychus urticae* Koch) infested tomato plants under greenhouse and laboratory conditions.**

Concentration (ppm)	No. of mites/leaf					
	Under greenhouse conditions			Under laboratory conditions		
	Pre-spray count	Post-spray count*	Reduction (%)**	Pre-spray count	Post-spray count*	Reduction (%)
Control	58.17	58.85	0.0 d	44.02	43.99	0.0 d
1000	45.20	34.18	24.20 c	37.65	27.22	27.70 c
2000	52.18	22.45	56.97 b	49.42	20.18	59.16 b
3000	49.94	11.01	77.95 a	66.34	12.12	81.73 a
4000	61.55	13.17	78.60 a	51.79	9.27	82.10 a

\*, counts made one week post treatment; \*\*, mortality values calculated with the Henderson-Tilton equation. Different letters in the vertical columns denote significant difference. (F-test,  $P < 0.05$ ,  $P < 0.01$ ).

**Table II.- Corrected mortality percentage of the predatory mite, *Neosiusulus cucumeris* Oudemans (Acari: Phytoseiidae) associated with tomato plants treated with four concentrations of Huwa-San TR50 under greenhouse and laboratory conditions.**

Concentration (ppm)	No. of predatory mites/leaf					
	Under greenhouse conditions			Under laboratory conditions		
	Average pre-spray count	Average post-spray count *	Reduction (%) **	Pre-spray count	Average post-spray count *	Reduction (%)
Control	9.85	9.75	0.0 d	11.44	11.43	0.0 d
1000	4.73	4.32	8.66 c	10.78	9.55	11.41 c
2000	9.64	8.41	12.75 b	7.29	6.07	16.73 b
3000	8.79	7.75	11.83 b	9.85	8.11	17.66 b
4000	12.85	7.91	38.44 a	8.88	5.14	42.11 a

\*, counts made one week post treatment; \*\*, mortality values calculated with the Henderson-Tilton equation. Different letters in the vertical column denote significant difference. (F-test,  $P < 0.05$ ,  $P < 0.01$ ).

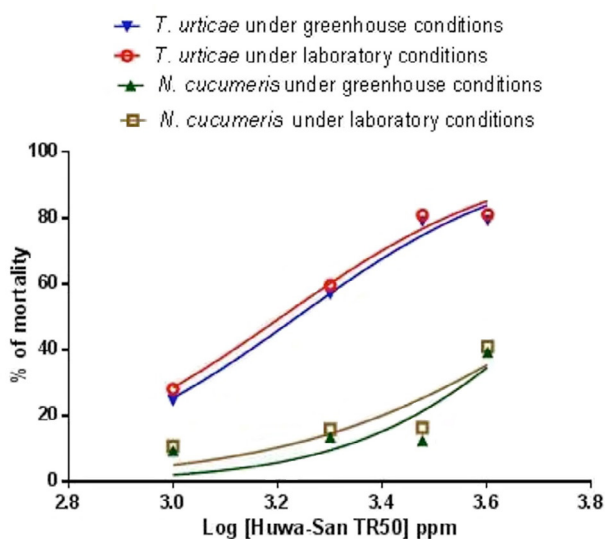


Fig. 1. Comparison of the average effects of Huwa-San TR50 on the mortality of two spotted spider mite, (*T. urticae* Koch) and the predatory mite (*N. cucumeris* Oudemans) after one week of exposure, expressed as a percentage of the control mortality in well water. Each point is the mean  $\pm$  SEM of 4 replicates, but in most cases, the error bars are smaller than the symbols used. The lines were fitted using a non-linear regression (log (inhibitor) vs. normalized response- variable slope) in Graphpad Prism 7 with the maximum plateau being 100% and the minimum being 0%. The  $IC_{50}$  values are listed in Table III.

**Table III.- The  $IC_{50}$  values for the effects of Huwa-San TR50 on both two spotted spider mite (*T. urticae* Koch) and the predatory mite, (*N. cucumeris* Oudemans) (Acari: Phytoseiidae) under laboratory conditions (LC) and greenhouse conditions (GC).**

Treatments	$IC_{50}$ (ppm) of Huwa-San TR50 (95% CI)
<i>T. urticae</i> under GC	1724 (1581 to 1891)
<i>T. urticae</i> under LC	1613 (1477 to 1762)
<i>N. cucumeris</i> under GC	5245 (3503 to 7853)
<i>N. cucumeris</i> under LC	5695 (3493 to 9284)

Under laboratory conditions, the mortality percentage was 24.20, 56.97, 77.95 and 78.60% for *T. urticae* and 8.66, 12.75, 11.83 and 38.44% for *N. cucumeris* at 1000, 2000, 3000 and 4000 ppm of Huwa-San TR50, respectively (Tables I and II). Under both conditions (greenhouse and laboratory), there was no significant difference between 3000 and 4000 ppm of Huwa-San TR50 on the mortality of *T. urticae*, whereas the mortality of *N. cucumeris* had an

insignificant difference at 2000 and 3000 ppm of Huwa-San TR50.

On the other hand, there was no significant difference between the two conditions (greenhouse and laboratory) ( $P = 0.9$  by using F-test in Graphpad Prism 7) on two spotted spider mite ( $P = 0.74$  by using F-test in Graphpad Prism 7) and on the predatory mite, *N. cucumeris* Oudemans, after one week of exposure to Huwa-San TR50 at all concentrations used (Fig. 1). Interestingly, Huwa-San TR50 did not produce any symptoms on tomato leaves up to 3000 ppm; whereas, 4000 ppm produced detectable leaf malformation after one week of treatment.

## DISCUSSION

Without doubt, pesticidal treatment can provide successful reduction in the pest population and protect the plant. Beyond this success however, many environmental impacts, toxicity to non-target species, residual effects and pest resistance can occur. Therefore, this has escalated scientific attention towards finding an alternative strategy for achieving a successful control with minimal side effects on non-target species. Alhewairini (2017) found that Huwa-San TR50 effectively killed cotton aphids (*A. gossypii*) and produced mortalities of 11.5, 42.5, 36.0 and 41.5% after 24 h and 93.5, 96.5, 97.0 and 95.5% after 48 h of exposure at 1000, 2000, 3000 and 4000 ppm, respectively. It also had no significant effects on honeybees and ladybird beetles. His findings showed that serious damage to the aphids' cuticle was produced after the Huwa-San TR50 application. Therefore, the experiments were designed to evaluate the toxic effect of Huwa-San TR50 on two spotted spider mite (*T. urticae* Koch) and the predatory mite (*N. cucumeris* Oudemans) under laboratory and greenhouse conditions. The obtained results are consistent with the previous study conducted by Alhewairini (2017), who found that Huwa-San TR50 produced a serious damage to the cuticle of *T. urticae* at the same concentrations, thereby resulting in its death. Furthermore, the cuticle damage of treated *T. urticae* was apparently stronger than that of the cotton aphids (*A. gossypii*), as the cuticle of most treated *T. urticae* were exploded. According to the literature review, these symptoms have never been recorded after application of all available insecticides and/or acaricides, which lead to the death of *T. urticae*.

The effects of the most available modern acaricides (e.g., abamectin, spiromesifen, chlorfenapyr and hexythiazox) can be produced through the effects on growth, development and disruption of respiratory processes (Dekeyser, 2005; Kramer and Schirmer, 2007). In contrast, Huwa-San TR50 can cause detectable symptoms that can be clearly seen in the cuticle of dead adults and nymphs.



Fig. 2. A, a non-treated adult and eggs of two spotted spider mite, *T. urticae* Koch (control); B, the cuticle damage of the treated *T. urticae* with 3000 ppm of Huwa-San TR50; C, treated and non-affected predatory mite, *N. cucumeris* Oudemans with 3000 ppm of Huwa-San TR50; D, non-hatching of eggs of *T. urticae* as compared with non-treated *T. urticae* (control).

According to the literature review, these symptoms have never been seen or reported previously, after applying the modern acaricides listed-above or other conventional acaricides. Moreover, these findings are consistent with the recent study conducted by [Alhewairini \(2017\)](#), who reported that 1000, 2000, 3000 and 4000 ppm of Huwa-San TR50 produced serious and observable damage to the cuticle of cotton aphids (*A. gossypii*) after 48 h of exposure. In addition, his findings pointed out that there was no statistically significant difference between these concentrations on the mortality after exposure for 48 h. In contrast, the highest mortality percentage of *T. urticae* and *N. cucumeris* was registered at 4000 ppm of Huwa-San TR50. However, there was no significant difference between 3000 and 4000 ppm in the mortality of *T. urticae* or *N. cucumeris* whether under greenhouse or laboratory conditions. Interestingly, at the highest concentration (4000 ppm), there was no damage on the cuticle of dead

*N. cucumeris*, as compared with *T. urticae*. This might be because predatory mites have dorsal and sternal shields. Nevertheless, the mode of action of Huwa-San TR50 is still unknown, although it has similar effects on the cuticle of *A. gossypii* and *T. urticae*.

The results of the current study indicated that Huwa-San TR50 affected all stages of *T. urticae* development, including the egg stage. In addition, the sharp reduction in the population of *T. urticae* was recorded after one week of treatment at 4000 ppm and produced mortalities of 82.10 and 78.60% under greenhouse and laboratory conditions, respectively. Apparently, the findings showed that *N. cucumeris* can feed on treated and/or dead *T. urticae* with Huwa-San TR50. Therefore, *N. cucumeris* can sustain its population on treated and/or dead *T. urticae*. Statistically, the difference between laboratory and greenhouse conditions was not significant ( $P > 0.5$ , using F-test Graphpad Prism 7).

Shah and Shukla (2014) evaluated the efficacy of abamectin against *T. urticae* on gerbera plants under polyhouse condition. It was found that abamectin decreased the population of *T. urticae* by 81.4%, after one week of application. Bernardi *et al.* (2012) tested the effects of abamectin and azadirachtin on *T. urticae* and predatory mites, *N. californicus* and *P. macropilis*, under laboratory conditions. They pointed out that azadirachtin and abamectin were effective against *T. urticae* and produced mortalities of 79 and 97%, respectively, after exposure for 72 h. However, abamectin was highly toxic to both predatory mites (*N. californicus* and *P. macropilis*) and produced mortalities of 60 and 85% after 72 h, respectively, whereas azadirachtin was less toxic and produced a mortality of 7% in both predatory mites. Abamectin has been extensively used in controlling pest insects and mites (Richard and Dybas, 1989), because it is considered safe under field condition, due to its fast degradation of surface residues, rapid uptake by plants and short environmental persistence (Kramer and Schirmer, 2007). In comparison with the above statement, up to 3000 ppm of Huwa-San TR50 reduced the population of *N. cucumeris* by 11.83 and 17.66% under greenhouse and laboratory conditions, respectively (Table II). Its reduction was much less than the reduction produced by abamectin and slightly higher than that produced by azadirachtin. However, 4000 ppm of Huwa-San TR50 reduced the *N. cucumeris* population by ~3-fold of the reduction gained at 3000 ppm in both conditions. Moreover, the maximum mortality was held between 3000 and 4000 ppm with no significant difference in the mortality of *T. urticae*.

Clearly, 3000 ppm seems to be very safe for the tomato plant and predatory mite (*N. cucumeris*) which is associated with the *T. urticae* infestation. Therefore, the application of 3000 ppm of Huwa-San TR50 on tomato plant is recommended.

On the other hand, field evaluation showed that up to 3000 ppm of Huwa-San TR50 produced leaf malformation of cucumber plants (Alhewairini, 2017). Huwa-San TR50 seems to have a variable sensitivity to the plant community. Therefore, it would be very useful to screen its sensitivity to different host plants of many pests.

## CONCLUSION

Further to the previous study conducted by Alhewairini (2017), it can be concluded that Huwa-San TR50 has shown acaricidal and pesticidal activities that can lead to the development of a new and safe strategy for controlling many pests. Huwa-San TR50 showed potential for use in the IPM program, as it had no significant effects on beneficial and predatory insects/mites, as compared

with the commonly and extensively used insecticides and acaricides. Furthermore, this potential makes the application of Huwa-San TR50 to be very suitable for use in combination with biological control agents. It would be very valuable to continue investigating the potential and effects on other predatory species associated with other host plants of *T. urticae*.

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### Statement of conflict of interest

Authors have declared no conflict of interest.

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