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Full Length Article

Huwa-San TR50 as an Acaricidal Agent in Controlling the Citrus Rust Mite, *Phyllocoptruta oleivora* (Ashmead) (Acari: Eriophyidae)

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Abstract

A field and laboratory study was conducted to investigate the effects of Huwa-San TR50, abamectin and bifenthrin on the citrus rust mite, *Phyllocopitruta oleivora* (Ashmead) (Acari: Eriophyidae). A marked reduction in the population of *P. oleivora* was seen after one week of exposure to Huwa-San TR50, abamectin and bifenthrin. The mortalities of *P. oleivora* were 91.02 and 94.12% for 6000ppm of Huwa-San TR50 (without observable damages on the surface of fruits and leaves), 79.27 and 81.74% for the recommended rate of abamectin and 82.12 and 84.00% for bifenthrin under field and laboratory conditions, respectively. Moreover, the hatching percentage of eggs of *P. oleivora* were 41.08 and 39.88% for 6000ppm Huwa-San TR50; and 96.23 and 95.86% for abamectin and 93.57 and 94.54% for bifenthrin under field and laboratory conditions, respectively. Statistically, the difference between the two conditions (field and laboratory) was insignificant ($P > 0.05$ by using F-test in Graphpad Prism 7) on both mortality and larvae hatching from eggs percentages of *P. oleivora*.

On the other hand, the population of the predatory mite, *Typhlodromips swirskii* was reduced by 31.78 and 31.97% for 6000ppm of Huwa-San TR50; and by 88.82 and 91.36% for abamectin and by 97.10 and 100% for bifenthrin under field and laboratory conditions, respectively. Thus, Huwa-San TR50 seems to be less toxic to the predatory mite, *T. swirskii*. These findings can support the implementation of Huwa-San TR50 as a new and safe acaricide agent for controlling *P. oleivora* in the integrated pest management (IPM) program.

Keywords: Huwa-San TR50; Citrus rust mite; *Phyllocoptruta oleivora*; *Typhlodromips swirskii*

Introduction

Citrus plants include a range of diverse fruit plants including oranges, lemons, grapefruits, pomelos and limes. They are one of the largest fruit crops in the world. Worldwide production and consumption of citrus fruit has grown rapidly since 1980 s. Current annual worldwide citrus production is estimated at over 70 million tons and half share to this is for oranges (Frederick and Xulan, 1990). Citrus production in Saudi Arabia is only in the region of Najran where total cultivation area is around 5000 hectares.

Citrus crops around the globe are susceptible to challenges including diseases caused by bacteria, fungi and insects. There are more than 3,690 species of eriophyoid mites and they are found around the globe (Amrine *et al*., 2003). Among the pest mites that strike citrus, is the citrus rust mite *Phyllocopitruta oleivora* (Ashmead) (Acari: Eriophyidae) (Silva *et al*., 2016). A significant citrus mite pest is the citrus rust mite, *P. oleivora* (Ashmead) (Acari: Eriophyidae). It is known to affect the quality and yield of citrus in humid tropical zones around the world, particularly when it reproduces rapidly under its preferred environmental conditions (Childers *et al*., 1996; Aghajanzadeh, 2003; Aghajanzadeh and Mallik, 2007). The major arthropod pest in Florida's citrus groves, responsible for the discoloration of fruit and the consequent loss of quality, is the citrus dust mite, *P. aleivora* (Ashmead) (Hall *et al*., 1991). *P. oleivora* has been reported on citrus in India, first in Karnataka (Puttarudraiah and Channabasavanna, 1959). America, Asia, Africa, Europe, and Australia are also home to this mite (Imbachi *et al*., 2012). It is endemic in orchards and has been slowly spreading. It feeds directly on the leaves, buds, shoots and fruit of the host plant (Manjunatha and Manjunatha, 2015). The results of *P. aleivora* are mummies and discolored and deformed fruit, and these cause changes to the production, quality, and appearance of the fruit (Mesa and Rodriguez, 2012).

There are more citrus rust mites found on the outer edges of the tree canopy's leaves and fruit during the summer months. They prefer the north bottom section of the tree and this is where the largest concentrations of mites can be found (Aghajanzadeh and Mallik, 2007). In mid-April for example, when young fruit begins to be available, the mites migrate to the fruit. The population is the

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highest on fruit during June, whereas the highest concentrations are on the leaves during the months of April and May (Knapp, 1994). Therefore, trap-catch of the mites was not affected in any consistent manner by compass direction (Bergh and McCoy, 1997). In addition, environmental factors, especially temperature and the amount of sunlight, lead citrus rust mites to mass within trees and on the fruit. They have also been found to cluster within lime trees. Thus, sampling is hampered by this aggregation (Hall *et al*., 1991).

Huwa-San TR50 began to be used about 20 years ago, and it is still frequently used as a disinfectant (www.huwasan.com). According to our literature studies, Huwa-San TR50 has never been used to control the citrus rust mite, *P. oleivora*.

Abamectin is a member of the macrocyclic lactonesa family, a product of a life form found in soil, first discovered in the mid-1970s (Lasota and Dybas, 1990). It frequently serves as an instecticide, an acaricide, and a nematicide, available under many brand names such as Vertimec, Reaper, Termictine 5% and CAM-MEK 1.8% EC. It is not persistent in the environment, so it does not accumulate.

Bifenthrin is a member of pyrethroid family and has been extensively used as an insecticide and an acaricide. It was found to be very toxic to fish and small aquatic organisms as well as bees (Johnson *et al*., 2010). It is sold under several trade names such as Transport, Maxxthor, Brigade, Talstar, Capture, Bifenthrine, Torant, Ortho Home Defense Max, Bifen IT, Bifen XTS, Bifen L/P, Zipak.

This study has been designed to (1) evaluate the potential of using Huwa-San TR50 as an acaricidal agent in controlling *P. oleivora*; (2) to test the side effects of Huwa-San TR50 on its predator, *T. swirskii* and (3) to evaluate the toxicity of acaricide (abamectin) and pyrethroid (bifenthrin) on *P. oleivora* and their side effects on its predatory *T. swirskii*.

Materials and Methods

Solutions and Experimental Protocol

Abamectin (Superbectine 3.6% w/v, abamectin) was obtained from Erzam company. Bifenthrin (Talstar 10% w/v, Bifenthrin) was obtained from Astra Company. The recommended application rates (25 mL/100 L for abamectin and 75 mL/100 L for Bifenthrin) for direct spray mixture were only used in this study.

Huwa-San TR50 was obtained from Ghatafan Company in Onaizah (retailer agent). Well water was used to dilute the liquid solution of Huwa-San TR50 (500,000 ppm) to give a serial concentration from 5000 to 6000 ppm. The study was conducted during the month of November 2017, under field and laboratory conditions.

The field experiments were carried out at the Experimental Research Station, Qassim University, Buraidah, Qassim, Saudi Arabia. Non-treated ten orange trees, *Citrus sinensis* were diagnosed with the infection of *P. oleivora* on their fruits. Twenty fruits were labeled from different branches and directions of each tree. Vaseline ring was put around the fruit peduncle to prevent mites from escaping. To record the pre-spray count, the total number of adults and eggs on each fruit was counted by using the magnifying glass. Thereafter, seven concentrations of Huwa-San TR50 and recommended rate of abamectin and bifenthrin were individually applied to each tree including control (well water) by using (10 L) knapsack sprayer. To record the post-spray count, the total number of adults and eggs on each fruit was counted by using the magnifying glass after one week of application.

Two hundred fruits were collected from non-treated ten orange trees, *Citrus sinensis* and then carefully placed in a clean plastic container and transported immediately to the laboratory for bioassay (25±2ºC and 70% relative humidity) at the Department of Plant Production and Protection, College of Agriculture and Veterinary Medicine, Qassim University to carry out laboratory experiments. The collected fruits were then distributed on 14 cm Petri dishes with a wet cotton as a background. To record the pre-spray count, the total number of adults and eggs on each fruit was counted by using binocular. To prevent mites from escaping, Vaseline ring was put on the bottom of the fruit. Thereafter, seven concentrations of Huwa-San TR50 and recommended rate of abamectin and bifenthrin were individually applied to each fruit including control (well water) by using (1 L) hand sprayer. To record the post-spray count, the total number of adults and eggs on each fruit was counted by using binocular after one week of application.

Statistical Analysis

Henderson and Tilton (1955) equation was used to determine the percentage of the citrus rust mite, *P. oleivora* and the predatory mite, *T. swirskii* population eradicated:

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

Where:

n = Number of *P. oleivora* or *T. swirskii*, T = Treated, Co = Control.

The percentage of *P. oleivora* or *T. swirskii* mortality was counted through direct observation. After that, Microsoft Excel was used to tabulate the average from this data and to figure out the percentage of the amount of larvae hatched from eggs. Statistically, all variables were examined through the use of one-way analysis of variance (ANOVA). Curves for the mortality assessments and number of larvae hatching from eggs were plotted using GraphPad Prism version 7. The data points were the mean ± 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 outcomes were expressed as mean mortality ± SEM for each treatment.

Results

The effect of Huwa-San TR50 on the citrus rust mite, *P. oleivora* was tested. Huwa-San TR50 showed high ability in killing *P. oleivora* under both field and laboratory conditions. This includes its ability in reducing the hatching percentage of eggs of *P. oleivora*.

Under field conditions, the mortality percentages of *P. oleivora* were 11.74, 29.68, 43.71, 52.83, 68.03, 88.02 and 91.02% whereas the percentages of larvae hatching from eggs of *P. oleivora* were 90.77, 80.01, 68.50, 53.04, 46.28, 42.20 and 41.08% after one week of exposure to 500, 1000, 2000, 3000, 4000, 5000 and 6000ppm of Huwa-San TR50, respectively (Table 1 and 2 and Fig. 1).

Under laboratory conditions, the mortality percentages of *P. oleivora* were 16.88, 35.58, 46.43, 57.33, 72.02, 90.48 and 94.12% whereas the percentages of larvae hatching from eggs of *P. oleivora* were 92.90, 82.42, 65.81, 55.35, 44.01, 43.65 and 39.88% after one week of exposure to 500, 1000, 2000, 3000, 4000, 5000 and 6000ppm of Huwa-San TR50, respectively (Table 1 and 2 and Fig. 1). Statistically, there was insignificant difference between the two conditions (field and laboratory) (*P* > 0.05 by using F-test in Graphpad Prism 7) on the mortality and larvae hatching from eggs percentages of *P. oleivora* (Table 6 and Fig. 1).

Side effects of Huwa-San TR50 on the predatory mite, *T. swirskii* (Athias-Henriot) was also tested. Huwa-San TR50 seems to have less toxicity to *T. swirskii* compared to *P. oleivora*. The mortality percentages were 2.50, 6.34, 14.80, 16.90, 18.03, 23.53 and 31.87% under field conditions and 4.21, 7.15, 15.50, 17.96, 18.75, 24.48 and 31.97% under laboratory conditions after one week of exposure to 500, 1000, 2000, 3000, 4000, 5000 and 6000 ppm of Huwa-San TR50, respectively (Table 3 and Fig. 1).

On the other hand, the effects of abamectin and bifenthrin on both mortality and number of larvae hatching from eggs of *P. oleivora* was tested. The mortality and larvae hatching from eggs percentages of *P. oleivora* were 79.27, 81.74% and 96.23, 95.86% for abamectin and 82.12, 84% and 93.57, 94.54% for bifenthrin after one week of exposure under field and laboratory conditions, respectively (Table 4). In addition, the mortality percentages of the predatory mite, were 88.82 and 91.36% for abamectin and 97.10 and 100.00% for bifenthrin after one week of exposure under field and laboratory conditions, respectively (Table 5). Statistically, there was insignificant difference between the effects of two acaricides (abamectin and bifenthrin) on both mortality and number of number of larvae hatching form eggs of *P. oleivora* (*P* > 0.05) but their effects were significantly different on the mortality of predatory mite, *T. swirskii* (*P* > 0.05) (Table 4 and 5).

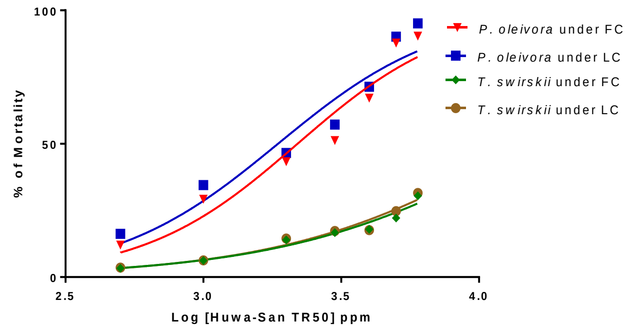

Fig. 1: Comparison of the average effects of seven concentrations of Huwa-San TR50 on the mortality of citrus rust mite, *P. oleivora* (Ashmead) (Acari: Eriophyidae) and the predatory mite, *T. swirskii* (Athias-Henriot) (Acari: Phytoseiidae) after one week of exposure, expressed as a percentage of the control mortality in well water under field conditions (FC) and laboratory conditions (LC). Each point is the mean ± SEM of 10 replicates, but in most cases, the error bars are smaller than the symbols used. The lines were fitted using a non-linear regression in Graphpad Prism 7 with the maximum plateau being 100% and the minimum being 0%

Discussion

Due to their nutritional value and health benefits, citrus fruits and their products are the part and parcel of our daily meal. The huge demand for citrus fruits, has inculcated farmers to focus on quality and yield of the crop. Therefore, farmers are going to use specialized pesticides to protect their crops and attain the highest yields. Extensive and excessive use of these chemicals for preventive and curative treatments has generated many issues, such as an increase in residual effects, environmental contamination and side effects on natural enemies as well as beneficial insects.

Traditional control of *P. oleivora* is based on chemicals like fenbutatin-oxid 550 SC, fenbutatin-oxid 500 SC, sulfur 80% and abamectin. These pesticides are effective in controlling *P. oleivora* when applied early enough at the rates normally advised (Abboud *et al.*, 2016). On the other hand, it has been found that most insecticides and acaricides are extremely poisonous to predatory mites such as abamectin, acetamiprid, lambda-cyhalothrin, chlorpyrifos and products containing pyrethrins (Fountain and Medd, 2015). Therefore, management of *P. oleivora* has taken the attention of researchers to find new acaricides which are very effective in controlling of *P. oleivora* with a minimal effect on its predators and the environment.

Very little research has been performed on the mite fauna found in Saudi Arabia and little has been done in the way of preparatory work for necessary taxonomic, ecological, and biological studies (Al-Atawi and Halawa, 2011). Although growing crops like dates, olives, and citrus are economically important to Saudi Arabia (Martin, 1972).

Table 1: Effect of seven concentrations of Huwa-San TR50 on citrus rust mite, *P. oleivora* (Ashmead) (Acari: Eriophyidae) infested orange trees *Citrus sinensis* under field and laboratory conditions

Concentration (ppm)	No. of mites/fruit					
	Under field conditions			Under laboratory conditions		
	Pre-spray count	Average post-spray count *	Mortality (%) **	Pre-spray count	Average post-spray count *	Mortality (%)**
Control	46.03	45.98	0.00 a	40.05	40.00	0.00 a
500	45.38	40.04	11.74 b	40.28	33.48	16.88 b
1000	43.79	30.78	29.68 c	43.73	28.17	35.58 c
2000	42.89	24.12	43.71d	50.11	26.84	46.43 d
3000	45.35	21.38	52.83 e	46.38	19.58	57.33 e
4000	43.92	12.79	68.03 f	51.74	14.48	72.02 f
5000	45.37	5.44	88.02 g	53.08	4.99	90.48 g
6000	42.42	3.69	91.02 g	48.15	2.82	94.12 g

*Average counts made one week post treatment
** Mortality values calculated with the Henderson-Tilton equation
Means followed by the same letter in a column are insignificantly different from each other at $P > 0.05$

Table 2: Number of protonymph hatching from eggs of the citrus rust mite, *P. oleivora* (Ashmead) (Acari: Eriophyidae) treated with seven concentrations of Huwa-San TR50 under field and laboratory conditions

Concentration (ppm)	No. of eggs and protonymph /fruit					
	Under field conditions			Under laboratory conditions		
	No. of eggs Pre-spray count	Average number of protonymph post-spray count *	Mortality (%) **	No. of eggs Pre-spray count	Average number of protonymph post-spray count *	Mortality (%) **
Control	64.35	65.02	0.00 a	60.88	61.10	0.00 a
500	66.27	60.17	90.77 b	59.32	55.13	92.90 b
1000	64.08	51.28	80.01 c	60.78	50.11	82.42 c
2000	67.29	46.11	68.50 d	70.28	46.27	65.81 d
3000	60.70	32.21	53.04 e	68.15	37.74	55.35 e
4000	61.25	28.36	46.28 f	66.91	29.45	44.01 f
5000	59.99	25.33	42.20 f	63.30	27.64	43.65 f
6000	67.35	27.68	41.08 f	65.00	25.94	39.88 f

*Average counts made one week post treatment
** Hatching percentage calculated with Excel Microsoft program
Means followed by the same letter in a column are insignificantly different from each other at $P > 0.05$

Table 3: Corrected mortality percentage of the predatory mite, *T. swirskii* (Athias-Henriot) (Acari: Phytoseiidae) associated with orange trees *Citrus sinensis* treated with seven concentrations of Huwa-San TR50 under field and laboratory conditions

Concentration (ppm)	No. of mites/fruit					
	Under field conditions			Under laboratory conditions		
	Pre-spray count	Average post-spray count *	Mortality (%) **	Pre-spray count	Average post-spray count *	Mortality (%)**
Control	5.22	5.20	0.00 a	4.87	4.80	0.00 a
500	5.11	4.98	2.50 b	4.75	4.55	4.21 b
1000	5.67	5.31	6.34 b	4.32	4.01	7.15 b
2000	6.31	5.42	14.80 c	5.54	4.68	15.50 c
3000	5.08	4.22	16.90 c	5.73	4.70	17.96 c
4000	5.76	4.72	18.03 c	6.34	5.15	18.75 c
5000	5.35	4.09	23.53 d	6.08	4.59	24.48 d
6000	5.55	3.78	31.87 e	5.72	3.89	31.97 e

*Average counts made one week post treatment
** Mortality values calculated with the Henderson-Tilton equation
Means followed by the same letter in a column are insignificantly different from each other at $P > 0.05$

Table 4: Effect of two acaricides on the mortality and number of larvae hatching from eggs of citrus rust mite, *P. oleivora* (Ashmead) (Acari: Eriophyidae) infested orange trees *Citrus sinensis* under field and laboratory conditions

Acaricides	Under field conditions						Under laboratory conditions					
	No. of mites/leaf			No. of eggs and larvae /leaf			No. of mites/leaf			No. of eggs and larvae /leaf		
	Pre-spray count	Average post-spray count *	Mortality % **	No. of eggs Pre-spray count	Average number of larvae post-spray count *	Hatching % ***	Pre-spray count	Average post-spray count *	Mortality % **	No. of eggs Pre-spray count	Average number of larvae post-spray count *	Hatching % ***
Control	44.39	44.40	0.00 a	30.14	30.22	0.00 a	46.37	46.25	0.00 a	31.25	31.00	0.00 a
Abamectin	45.28	9.38	79.27 b	31.05	29.88	96.23 b	46.66	8.25	81.74 b	32.83	31.48	95.86 b
Bifenthrin	44.92	8.02	82.12 b	32.18	30.12	93.57 b	45.28	7.24	84.00 b	30.17	28.53	94.54 b

* Average counts made one week post treatment
** Mortality values calculated with the Henderson-Tilton equation
*** Hatching percentage calculated with Excel Microsoft program
Mean followed by the same letter in a column are insignificantly different from each other at $P > 0.05$

Table 5: Corrected mortality percentage of the predatory mite, *T. swirskii* (Athias-Henriot) (Acari: phytoseiidae) associated with orange trees *Citrus sinensis* treated with two pesticides under field and laboratory conditions

Acaricides	No. of mites/fruit					
	Under field conditions			Under laboratory conditions		
	Pre-spray count	Average post-spray count *	Mortality (%) **	Pre-spray count	Average post-spray count *	Mortality (%)**
Control	4.89	4.99	0.00 a	5.18	5.20	0.00 a
Abamectin	5.02	0.56	88.82 b	5.34	0.46	91.36 b
Bifenthrin	4.86	0.14	97.10 c	4.75	0.00	100.00 c

* Average counts made one week post treatment
** Mortality values calculated with the Henderson-Tilton equation
Mean followed by the different letter in a column are significantly different from each other at $P < 0.05$

Table 6: Shows the IC_{50}values for the effects of Huwa-San TR50 on both Citrus Rust Mite *P. oleivora* (Ashmead) (Acari: Eriophyidae) and the predatory mite, *T. swirskii* (Athias-Henriot) (Acari: Phytoseiidae) under field conditions (FC) and laboratory conditions (LC)

Treatments	IC_{50} (ppm) of Huwa-San TR50 (95% CI)
P. oleivora under FC	2197 (1922 to 2486)
P. oleivora under LC	1867 (1601 to 2149)
T. swirskii FC	16368 (13163 to 21676)
T. swirskii LC	14757 (12046 to 19173)

* Calculated by using F-test in Graphpad Prism 7

This study has been designed to evaluate the potential of using Huwa-San TR50 as an acaricidal agent in controlling *P. oleivora* and predatory mite *T. swirskii* found in citrus orchards of Saudi Arabia. Huwa-San TR50 is a mixture of hydrogen peroxide (H_2O_2) in water to which traces of silver ions (Ag^+) are added to stabilize H_2O_2. Upon decomposition, it produces water (H_2O) and atomic oxygen which is spontaneously converted to atmospheric O_2 (Roam Technology, www.huwasan.com). Huwa-San TR50 is therefore, healthy to life and friendly to environment contrary to the traditional pesticides. The authors of this paper have already reported, successful use of Huwa-San TR50 on several acari pests like date palm mite (*O. afrasiaticus)*, Varroa mite (*V. jacobsoni)*, two-spotted spider mite (*T. urticae*) and tomato russet mite (*A. lycopersici*) (Alhewairini and Al-Azzazy, 2017a, b; Al-Azzazy and Alhewairini, 2018a, b).

Huwa-San TR50 had significant effects on both the mortality and the number of larvae hatching from eggs of *P. oleivora*. Mortality of *P. oleivora* has been found to be associated with the concentration of Huwa-San TR50 because at concentrations of 500, 1000, 2000, 3000, 4000, 5000 and 6000 ppm, a decline in the population of *P. oleivora* was 11.74, 29.68, 43.71, 52.83, 68.03, 88.02 and 91.02% under field conditions and by 16.88, 35.58, 46.43, 57.33, 72.02, 90.48 and 94.12% under laboratory conditions, respectively (Table 1). After application of Huwa-San TR50, no damage was seen on the surface of fruits or on the leaves at all concentrations used in this study.

Abboud *et al.* (2016) reported the use of traditional acaricides; fenbutatin-oxid 550 SC, fenbutatin-oxid 500 SC, sulfur 80% and abamectin. Their mortality results are comparable to our results obtained with Huwa-San TR50 as abamectin can reduce the population of *P. oleivora* by 79.27 and 81.74% under field and laboratory conditions, respectively (Table 4). In comparison with these findings, Huwa-San TR50 is more effective than the traditional acaricides (abamectin and bifenthrin, (Table 1 and 4) because it is fatal to phytophagous mite and relatively safe to the predatory mite, *T. swirskii* (Table 5). Furthermore, bifenthrin was found to be significantly toxic to the predatory mite *T. swirskii* than abamectin ($P < 0.05$), although there was an insignificant difference in their effects on the population of *P. oleivora* as well as the number of larvae hatching form eggs ($P > 0.05$) (Table 5 and Fig. 1).

It might be argued that predatory mites obtained from commercial companies are resistant to many acaricides or/and insecticides and they can sustain their populations better than natural predatory mites. This is without doubt true, but in most cases natural predatory mites are naturally associated with their prey and they will be in direct contact of acaricides or/and insecticides. In addition, predator recovery can take some time, allowing the revitalization of the population of the pests (Fountain and Medd, 2012). Therefore, care must be taken to maintain populations as much as possible.

In summary, Huwa-San TR50 is a better replacement of traditional acaricides because it is significantly toxic to *P. oleivora* (citrus mite), supportive to biological control and safe to the environment.

Conclusion

This study has proven that Huwa-San TR50 can be used as a safe alternative acaricide in controlling the citrus rust mite, *P. oleivora*, as treatment with Huwa-San TR50 showed the best control of *P. oleivora*. Huwa-San TR50 treatment can also spare *T. swirskii* which is associated with the infection of *P. oleivora*, whereas the recommended rate of abamectin and bifenthrin treatment can destroy more than 90% of the population of *T. swirskii*.

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