



Effectiveness of Huwa-San TR50 on Tomato Russet Mite *Aculops lycopersici* (Masse) (Acari: Eriophyidae)

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ABSTRACT

This study evaluated the effectiveness of Huwa-San TR50 against all stages of *Aculops lycopersici*. This includes its side effects on the associated predatory mite, *Neosiulus cucumeris*. Huwa-San TR50 is a formulation of hydrogen peroxide, stabilized by the addition of a small quantity of silver and is extensively used as a disinfectant. It was found to be very effective in killing *A. lycopersici* with no significant effect on *N. cucumeris*. Under greenhouse conditions, the mortality percentages were 81.17, 84.11, 92.74 and 95.10% for *A. lycopersici* and 15.86, 23.45, 33.33 and 58.19% for *N. cucumeris* after one week of exposure to 1000, 2000, 3000 and 4000ppm of Huwa-San TR50, respectively. Under laboratory conditions, the mortality percentages were 83.24, 85.13, 94.21 and 97.36% for *A. lycopersici* and 17.14, 27.55, 37.14 and 59.37% for *N. cucumeris* at 1000, 2000, 3000 and 4000 ppm of Huwa-San TR50, respectively. In addition, Huwa-San TR50 caused a concentration dependent reduction in the percentage of egg hatching. The percentages of larvae hatching from eggs of *A. lycopersici* were 73.42, 56.92, 25.41 and 17.97 under greenhouse conditions and 68, 53.14, 23.51 and 15.18 under laboratory conditions at 1000, 2000, 3000 and 4000ppm of Huwa-San TR50 after one week of treatment compared with the control (well water), respectively. The application of Huwa-San TR50 showed a promising safety profile when compared to other acaricides, as it spared predatory mites at concentrations which had a high lethality to tomato russet mites. Hence, Huwa-San TR50 can be utilized in the Integrated Pest Management (IPM) program as an acaricide agent.

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Authors' Contribution

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

Key words

Huwa-San TR50, Tomato russet mite, *Aculops lycopersici*, *Neosiulus cucumeris*.

INTRODUCTION

Tomato russet mite (TRM), *Aculops lycopersici* (Masse) (Acari: Eriophyidae) was first described in Australia by Masse (1937). Thereafter, it was recorded on tomato plants in several countries such as Korea (Kim *et al.*, 2002), USA (Anderson, 1954), Egypt (Abou-Awad, 1979), Argentina (Rossi, 1962), Brazil (Fletchman and Aranda, 1970), Venezuela (Cermeu *et al.*, 1982), Japan (Nemoto, 2000) and Saudi Arabia (Gentry, 1965; Martin, 1971; EPPO, 2014). It was considered as an important pest of tomato (*Solanum lycopersicum* L.) because it can cause serious damage to tomato plants. Its infestations cause destruction of the epidermal cells of the leaflet leading to a curling of the leaflet edges, desiccation, tissue damage and plant death (Keifer *et al.*, 1982; Royalty and Perring, 1988; Haque and Kawai, 2002). It can also rust or cross-crack the surface

of fruits (Kim *et al.*, 2002). Moreover, there are more than 24 host plants of *A. lycopersici* distributed in three different plant families Solanaceae, Convolvulaceae and Rosaceae (Perring, 1996; Larrain, 2000; Duso *et al.*, 2010).

The efficacy of several insecticides and/or acaricides against *A. lycopersici* has been tested. For example, Abou-Awad and El-Banhawy (1985) evaluated the susceptibility of *A. lycopersici* to methamidophos, pyridaphenthion, cypermethrin, dicofol and fenarimol. In addition, Royalty and Perring (1987) compared the toxicity of five acaricides namely avermectin B1, dicofol, cyhexatin, sulfur, and thuringiensin to *A. lycopersici*. Kashyap *et al.* (2014) tested the efficacy of abamectin, azadirachtin, spiromesifen, mineral oil and hexythiazox against *A. lycopersici*.

In comparison with mite species belonging to Tetranychidae, Eriophyoid mites showed equal susceptibility to many acaricides such as amitraz, dicofol, propargite, ethion, bromopropylate and abamectin (Thomas *et al.*, 2009). However, Tetranychidae suffered few effects from benzoylphenylurea insecticides diflubenzuron and teflubenzuron whereas russet mites were adversely

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affected (Childers *et al.*, 1996; Scarpellini and Clari, 1999). Despite the high susceptibility of Eriophyoid mites to most available acaricides, several species of Eriophyoid mites (including *A. lycopersici*) have developed resistance. In Egypt for example, the resistance of *A. lycopersici* to methamidophos was reported after three seasons of use (Abou-Awad and El-Banhawy, 1985). This would encourage scientific researchers to find alternative strategies for controlling *A. lycopersici*.

Little information has been published on the control of *A. lycopersici* and Huwa-San TR50 has not been considered for use against this species of mite. Alhewairini (2017) was the first to report that Huwa-San TR50 can be used as an insecticide in controlling cotton aphids (*Aphis gossypii* Glover) without significant effects on honeybees (*Apis mellifera lamarckii*) and seven-spot ladybird beetles (*Coccinella septempunctata*). Moreover, Alhewairini and Al-Azzazy (2017a, b, c) were the first to document that Huwa-San TR50 has acaricidal activity as it can successfully kill two spotted spider mites (*Tetranychus urticae* Koch) with minimal effects on its associated predatory mite, (*Neosiusulus cucumeris*), *Varroa* mite (*Varroa jacobsoni* Oudemans) and the date palm mite, *Oligonychus afrasiaticus* (McGregor).

Huwa-San TR50 is widely used as a disinfectant and was developed over twenty years ago (www.huwasan.com). It is a formulation of hydrogen peroxide which has been stabilized by the addition of a small quantity of silver (www.huwasan.com). Furthermore, it has 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).

This study evaluated the effectiveness of Huwa-San TR50 against *A. lycopersici* and its secondary effects on the associated predatory, *N. cucumeris* under greenhouse and laboratory conditions.

MATERIALS AND METHODS

Solutions and experiment protocol

Huwa-San TR50 was obtained from Ghatafan Company in Onaizah (retailer agent). The field experiments were carried out at the Experimental Research Station, Qassim University, Buraidah, Al-Qassim, Saudi Arabia under greenhouse conditions during the May 2017 season in an abandoned tomato (*Solanum lycopersicum* L.) greenhouse, having a history of tomato russet mite infestations. 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.

About 10 m² of cultivated land with tomato seedlings (Pritchard cultivar) was chosen and divided into 5 plots (each plot was about 2 m²), and all plots were arranged using a randomized complete block design. Ten tomato leaves of each treatment were randomly collected and placed in a clean labeled plastic bag and transported to the laboratory to determine the initial density and distribution of *A. lycopersici* and *N. cucumeris* (moving stages and eggs) as a pre-spray count using a stereomicroscope. Under greenhouse conditions, four different concentrations 1000, 2000, 3000 and 4000 ppm of Huwa-San TR50 including control (well water) with 5 replicates were directly sprayed on the tomato plants (heavily infested by tomato russet mite) using a knapsack sprayer (20 L).

Direct observations were made one week after the application of four Huwa-San TR50 concentrations, by using a stereomicroscope to determine the percentage reduction in the population of tomato russet mites on the tomato plants after spraying.

The laboratory experiments were conducted in a laboratory (25±2°C and 70% relative humidity). Non-treated tomato leaves (heavily infested by tomato russet mite) were randomly collected and placed in a clean plastic bag and then transferred to the Department of Plant Production and Protection, College of Agriculture and Veterinary Medicine, Qassim University for immediate bioassay. The collected tomato leaves were arranged in a randomized complete block design.

Wet cotton, 4.0 cm in diameter, was used as rearing arenas in plastic Petri-dishes (5 × 1 cm) with the lower surface of leaves facing upwards. The cotton bed was kept wet by soaking with distilled water twice daily to keep the leaf discs fresh and to avoid the migration of mites to the lower leaf surface. The leaf discs were checked under a stereomicroscope to ensure that no other predatory insects and mites were present. To determine pre-spray counts, the number of *A. lycopersici* and *N. cucumeris* (moving stages and eggs) was manually counted by direct observation under a stereomicroscope. Thereafter, four different concentrations 1000, 2000, 3000 and 4000 ppm of Huwa-San TR50 including control (well water) with 5 replicates were sprayed directly on the Petri-dishes by using a small knapsack sprayer (1L). Dead mites were counted one week after applying control (well water) and four concentrations of Huwa-San TR50 to determine the percentage mortality.

Statistical analysis

The percentage reduction in the average populations of tomato russet mite (*A. lycopersici*) and the predatory mite (*N. cucumeris* Oudemans) were calculated using the equation of Henderson and Tilton (1955).

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

Where, n is number of *A. lycopersici* and *N. cucumeris* population, T is treated, Co is control and Tr is treatment.

The mortalities of *A. lycopersici* and *N. cucumeris* were calculated manually by direct observation under a stereomicroscope. Thereafter, the average percentage of a number of larvae hatching from eggs was calculated by using Microsoft Excel Program. All variables of the obtained data were statistically analyzed using One-way analysis of variance (ANOVA). Curves for the mortality assays and number of larvae hatching from eggs 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 with a maximum and a minimum plateau. The results are expressed as mean mortality percentage \pm SEM for each treatment.

RESULTS

The obtained results showed the effectiveness of

Huwa-San TR50 against all stages of *A. lycopersici* including eggs with a minimal effect on the mortality of the associated predatory *N. cucumeris*.

Under greenhouse conditions, the mortality percentages were 81.17, 84.11, 92.74 and 95.10% for *A. lycopersici* and 15.86, 23.45, 33.33 and 58.19% for *N. cucumeris* one week after exposure to 1000, 2000, 3000 and 4000 ppm of Huwa-San TR50, respectively (Tables I, II). Under laboratory conditions, the mortality percentages were 83.24, 85.13, 94.21 and 97.36% for *A. lycopersici* and 17.14, 27.55, 37.14 and 59.37% for *N. cucumeris* at 1000, 2000, 3000 and 4000 ppm of Huwa-San TR50, respectively (Tables I, II). The maximum mortality (> 95%) was obtained after exposure to 4000 ppm of Huwa-San TR50; although the difference between 3000 and 4000 ppm of Huwa-San TR50 on the mortality of *A. lycopersici* was statistically insignificant in both conditions (greenhouse and laboratory) (Table I). Clearly, Huwa-San TR50 affects the color of the eggs and cuticle of adult *A. lycopersici*, by changing them to light brown from white and by inducing malformation of the eggs and the cuticle of the adult.

Table I.- Effect of four concentrations of Huwa-San TR50 on tomato russet mite *Aculops lycopersici* (Masse) (Acari: Eriophyidae) infested tomato plants under greenhouse and laboratory conditions.

| Concentration (ppm) | No. of mites/leaf | | | | | |
|---------------------|-----------------------------|----------------------------|-----------------|-----------------------------|----------------------------|-----------------|
| | Under greenhouse conditions | | | Under laboratory conditions | | |
| | Pre-spray count | Average post-spray count * | Reduction (%)** | Pre-spray count | Average post-spray count * | Reduction (%)** |
| Control | 101.14 | 99.23 | 0.00 a | 88.56 | 87.45 | 0.00 a |
| 1000 | 104.85 | 18.24 | 81.17 b | 87.45 | 14.82 | 83.24 b |
| 2000 | 90.11 | 14.58 | 84.11 b | 82.64 | 12.37 | 85.13 b |
| 3000 | 102.78 | 8.13 | 92.74 c | 91.69 | 5.28 | 94.21 c |
| 4000 | 98.71 | 4.02 | 95.10 c | 86.38 | 2.52 | 97.36 c |

*Counts made one week post treatment. ** Mortality values calculated with the Henderson-Tilton equation. Mean followed by the same letter in a column are not significantly different from each other at $P>0.05$.

Table II.- Corrected mortality percentage of the predatory mite, *Neosiulus 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 | | |
| | Pre-spray count | Average post-spray count* | Reduction (%)** | Pre-spray count | Average post-spray count* | Reduction (%)** |
| Control | 5.12 | 5.25 | 0.00 a | 4.98 | 4.89 | 0.00 a |
| 1000 | 5.73 | 4.81 | 15.86 b | 4.55 | 3.77 | 17.14 b |
| 2000 | 6.13 | 4.66 | 23.45 c | 4.56 | 3.29 | 27.55 c |
| 3000 | 6.03 | 4.00 | 33.33 d | 5.17 | 3.21 | 37.14 d |
| 4000 | 5.81 | 2.41 | 58.19 e | 5.88 | 2.31 | 59.37 e |

*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 III.- Number of larvae hatching from eggs of the tomato russet mite *Aculops lycopersici* (Masse) (Acari: Eriophyidae) treated with four concentrations of Huwa-San TR50 under greenhouse and laboratory conditions.

| Concentration (ppm) | No. of eggs and larvae /leaf | | | | | |
|---------------------|------------------------------|--|-----------------|-----------------------------|--|-----------------|
| | Under greenhouse conditions | | | Under laboratory conditions | | |
| | No. of eggs pre-spray count | Average number of larvae post-spray count* | Hatching (%) ** | No. of eggs pre-spray count | Average number of larvae post-spray count* | Hatching (%) ** |
| Control | 34.45 | 34.22 | 0.00 a | 36.78 | 35.67 | 0.00 a |
| 1000 | 34.36 | 25.23 | 73.42 b | 35.47 | 24.12 | 68.00 b |
| 2000 | 36.87 | 20.99 | 56.92 c | 37.18 | 19.76 | 53.14 c |
| 3000 | 39.82 | 10.12 | 25.41 d | 35.17 | 8.27 | 23.51 d |
| 4000 | 40.28 | 7.24 | 17.97 e | 34.58 | 5.25 | 15.18e |

*Counts made one week post treatment. ** Hatching percentage calculated with Microsoft Excel program. Mean followed by the different letter in a column are significantly different from each other at $P < 0.05$.

There was no significant difference between the two conditions (greenhouse and laboratory) on the mortality of *A. lycopersici* ($P = 0.713$ by using F-test in Graphpad Prism 7) and the predatory mite, *N. cucumeris*, ($P = 0.623$ by using F-test in Graphpad Prism 7) after one week of exposure to Huwa-San TR50 at all concentrations used (Fig. 3). However, the difference between tomato russet mite (*A. lycopersici*) and the predatory mite (*N. cucumeris* Oudemans) at the two conditions (greenhouse and laboratory) was significant ($P < 0.01$ by using F-test in Graphpad Prism 7) (Fig. 1).

The percentages of larvae hatching from the eggs of *A. lycopersici* were 73.42, 56.92, 25.41 and 17.97 under greenhouse conditions and 68, 53.14, 23.51 and 15.18 under laboratory conditions at 1000, 2000, 3000 and 4000 ppm of Huwa-San TR50 after one week of treatment compared with control (well water), respectively (Table III).

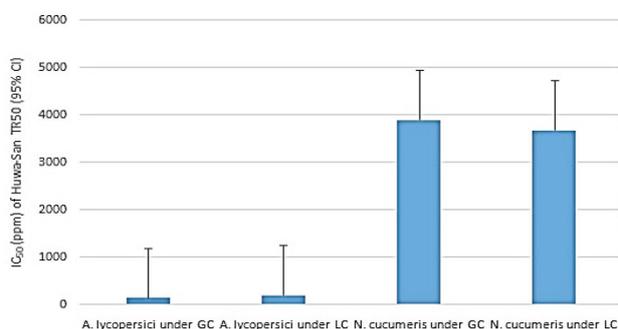


Fig. 1. The difference between the IC_{50} values for the effects of Huwa-San TR50 on both tomato russet mite *A. lycopersici* and the predatory mite, *N. cucumeris* Oudemans) (Acari: Phytoseiidae) under laboratory conditions (LC) and greenhouse conditions (GC).

DISCUSSION

Unfortunately, many acarids including *A. lycopersician* sustain their population and become insensitive to the regular application of most available acaricides due to their resistance to these acaricidal agents. Researchers are interested in finding alternative acaricidal agents that can effectively be used in controlling *A. lycopersici*. Therefore, this study was designed to test the effectiveness of Huwa-San TR50 against the adult and eggs of *A. lycopersici* including its side effects on the associated predatory mite, *N. cucumeris* under both greenhouse and laboratory conditions.

The laboratory results of this study showed that the application of Huwa-San TR50 caused serious damage to the cuticle of *A. lycopersici*. Its application can also turn the eggs and cuticle color of adult *A. lycopersici* to from white to light brown as well as causing malformation of the egg shape and adult cuticle. This damage resulted in the death of *A. lycopersici* and a failure in the hatching of eggs. These findings are consistent with previous findings already reported by Alhewairini and Al-Azzazy (2017a, b, c). Huwa-San TR50 showed the same damage symptoms on the cuticle of *A. lycopersici* which were already seen on two spotted spider mites (*T. urticae*), *Varroa* mite (*V. jacobsoni*) and the date palm mite (*O. afrasiaticus*). Moreover, similar damage to the cuticle were seen on cotton aphids (*A. gossypii*) (Alhewairini, 2017) and after the application of a certain concentration of Huwa-San TR50.

More than 99% mortality of *A. lycopersici* was achieved by applying azadirachtin, spiromesifen, mineral oil, hexythiazox and abamectin (Kashyap *et al.*, 2014). The latter was the most toxic among these acaricides. In

comparison with this statement, 3000 ppm of Huwa-San TR50 was reported to be safe to tomato leaves (Alhewairini and Al-Azzazy, 2017a). At this concentration, 95.10% of *A. lycopersici* was killed and only 25.41% of the exposed eggs were successfully hatched. Clearly, Huwa-San TR50 seems capable of disrupting the eggs hatching of *A. lycopersici*, because the exposed eggs failed to hatch when the concentration of Huwa-San TR50 was increased (Table II; Fig. 2).

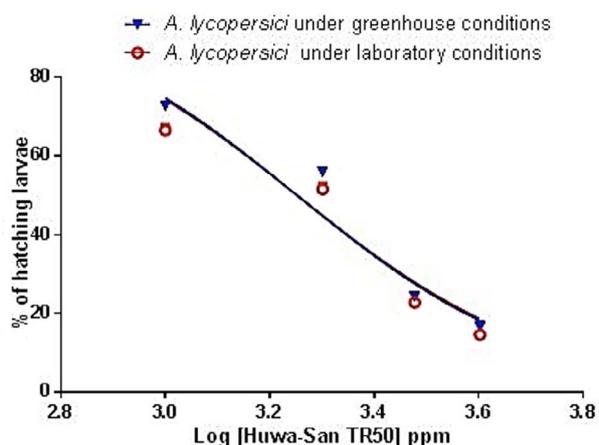


Fig. 2. Effects of Huwa-San TR50 on the average mortality on larvae hatching from eggs of the tomato russet mite under greenhouse and laboratory conditions after one week of exposure, expressed as a percentage of the control mortality in well water. Each point is the mean \pm SEM of 5 replicates, but 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 80% and the minimum being 0%.

Interestingly, 3000 ppm had an insignificant effect on the mortality of the associated predatory mite, *N. cucumeris* (< 33%); although it was exposed to Huwa-San TR50 by direct application on treated *A. lycopersici*. This emphasizes the selectivity of Huwa-San TR50 against both the adult and eggs of *A. lycopersici*. Furthermore, 3000 ppm of Huwa-San TR50 was less toxic to the predatory mite, *N. cucumeris* by about 24% compared with 4000 ppm under both conditions (greenhouse and laboratory). Therefore, there is no need to exceed 3000 ppm of Huwa-San TR50 when controlling *A. lycopersici*, since leaf malformation of tomato plants was already registered beyond this concentration (Alhewairini and Al-Azzazy, 2017a). In contrast, abamectin was found to be highly toxic to both predatory mites (*Neosiulus californicus* and *Phytoseiulus macropilis*) and produced mortalities of 60 and 85% after 72 h, respectively (Bernardi et al., 2012).

Therefore, the control of *A. lycopersici* with Huwa-San TR50 would most unlikely reduce the population number of the associated predatory mite, *N. cucumeris* compared with most available acaricides.

The results obtained are consistent with our previous results as Huwa-San TR50 was found to be very safe to predatory mite, *N. cucumeris* (Alhewairini and Al-Azzazy, 2017a). In another study, Huwa-San TR50 was also non-toxic to beneficial insects such as honeybees (*Apis mellifera lamarekii*) up to 4000 ppm and seven-spot ladybird beetles (*Coccinella septempunctata*) up to 3000 ppm (Alhewairini, 2017). This means that Huwa-San TR50 can effectively kill and cause a failure in egg hatching of *A. lycopersici* with insignificant effect on the number of predatory mite and insects.

Finally, Huwa-San TR50 provided an effective suppression of *A. lycopersici* population as it can effectively kill and produce a failure in the eggs hatching of *A. lycopersici* under both greenhouse and laboratory conditions.

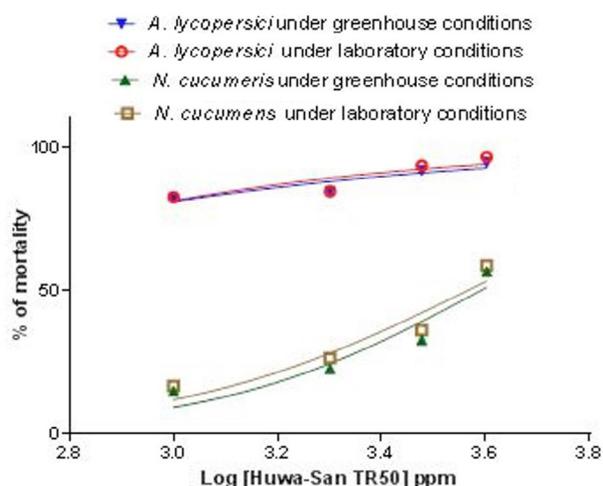


Fig. 3. Effects of Huwa-San TR50 on the average mortality of tomato russet mite *A. lycopersici* and the predatory mite (*N. cucumeris* Oudemans) after one week of exposure, expressed as a percentage of the control mortality in well water. Each point is the mean \pm SEM of 4 replicates, but in most cases, 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 110% and the minimum being 0%.

CONCLUSION

This study has determined a new target organism that can be effectively killed by the application of Huwa-San

TR50. The application of Huwa-San TR50 causes damage to the cuticle of *A. lycopersici* leading to the death of this mite. This includes its ability to reduce the number of larvae hatching from eggs which can help in achieving successful control by reducing the mite population. Further research is encouraged to investigate the mode of action of Huwa-San TR50 on the cuticle of tomato russet mite (*A. lycopersici*). The application of Huwa-San TR50 showed a promising safety profile when compared to other acaricides, since it spared a reasonable number of predatory mites. Hence, Huwa-San TR50 can be used in the IPM program as an acaricidal agent.

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Statement of conflict of interest

The authors declare that there is no conflict of interest.

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