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Laboratory Evaluation of the Toxicity of Oxamyl against the Date Palm Mite, *Oligonychus afrasiaticus* (McGregor) (Acari:Tetranychidae)

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ABSTRACT

The toxicity of oxamyl against the date palm mite, *Oligonychus afrasiaticus* (McGregor) was evaluated. This includes the potential of Injo200 and Huwa-San TR50 in increasing the effectiveness of oxamyl against all developmental stages including eggs of *O. afrasiaticus*. Oxamyl was found to be very effective in controlling the adult of *O. afrasiaticus* with a little effect on its eggs under laboratory conditions. The mortality percentages of *O. afrasiaticus* were 61.79, 73.58 and 82.90%; 62.87, 73.77 and 83.32%; 71.70, 82.23 and 92.37% after one week of exposure to the quarter recommended dose (QRD), half recommended dose (HRD) and recommended dose (RD) of oxamyl only and with the recommended dose (RD) of Injo200 or 1000ppm of Huwa-SanTR50, respectively. The percentages of larvae hatching from eggs of *O. afrasiaticus* were 97.20, 94.62 and 85.53%; 94.70, 93.32 and 84.86%; 54.01, 57.93 and 46.21% after one week of exposure to QRD, HRD and RD of oxamyl only and with Injo200 or 1000ppm of Huwa-SanTR50, respectively. Adding 1000ppm of Huwa-SanTR50 to QRD, HRD and RD of oxamyl was found to significantly increase the toxicity of oxamyl to all developmental stages of *O. afrasiaticus*, whereas, the RD of Injo200 had no significant toxic effect on *O. afrasiaticus* with or without oxamyl. This does not mean that Injo200 is useless but it might be very effective on other pests rather than *O. afrasiaticus*. Finally, this study has provided a new acaricide that can be effectively used in controlling *O. afrasiaticus*. This includes the new approach of mixing Huwa-San TR50 with oxamyl which significantly increases its effectiveness against *O. afrasiaticus*. This approach can help not only in reducing control cost but also in reducing residual effects of acaricides on date fruits.

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INTRODUCTION

The old world date mite or the date palm mite, *Oligonychus afrasiaticus* (McGregor) has attained the status of an economically important pest affecting the date palm, *Phoenix dactylifera,* as it attacks and causes devastation to the date palm crop in all its developmental stages. This pest has caused havoc to the date palm fruits industry in Saudi Arabia. Consequently, it is considered the most destructive to date fruits in the kingdom that had more than 28 million date trees in 2015 (General Authority for Statistics KSA, 2015). The pest can develop under a variety of environmental conditions but dryer environmental places provide more conducive conditions for it to flourish. It produces six generations a year (Hussain, 1969). Infestation by the date palm mite causes significant and extensive yield loss as 100% yield loss was already recorded as a result of its infestation (Arbabi *et al*., 2006, 2010). In Iraq for example, 50-80% yield loss was caused by the infestation of date palm mite even in dry, dusty and stormy weather conditions (Al-Jboory and Al-Suaide, 2010). During its infestation, it builds dusty and creamy silken webs on date bunches and around date fruits. *O. afrasiaticus*'s attack makes date bunches and date fruits more vulnerable to catch dust and sand grains which in turn provides a conducive environment for the production and development of the pest. This phenomenon causes change in the colour of date fruits from green to silvery-white and they exhibit a dusty appearance (Saleh and Hosny, 1979). It was also recorded by Ali and Aldosari (2007) that the significant positive correlation was found between injury rates by *O. afrasiaticus* and protein content in date palm fruits. Consequently, the date fruit loses their value by becoming unsuitable for human consumption. The only remedial strategy to inhibit the growth of *O. afrasiaticus* being used by the date palm growers is the use of chemicals. In Saudi Arabia, Iraq and Iran, several previous studies were conducted to evaluate the efficacy of a wide range of acaricides against the date palm mite, under laboratory and field conditions. This includes Tedion (Tetradifon 75.2 g L-1), Kelthane (Dicofol 18.5 EC), Neoron (Bromopropylate 25% EC), Ekatin (Thiometon 25% EC), Microthiol special (Sulfur 80%WP), Top cop (73% S + 6.4% Cu, w/v), Transact (Abamectin 1.8% EC)

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and Peropal (Azocyclotin 25% WP) Albarq (Triazophos 40% EC and Deltamethrin 2.5% EC), Baythroid (Cyfluthrin 5% EC), Lord (Chlofenapyr 24% SC), Final (Alpha cypermethrin 10% EC) and Falcon (Triazophos), Vertamic 1.8% EC, Salocide 40% EC, Amitraz 20% EC, Perpol 25% WP, Fenpropathrin 10% EC, Fenpyroximate 5% SC, Fenazaquin 20% SC, Propargite 57% EC, Tetradifon 18.5% EC and Hexythiazox 10% as well as colour traps (e.g. white, yellow, green, blue and red) against *O. afrasiaticus* (Al-Doghairi, 2004; Al-Dosary, 2010; Aldosari, 2009; Arbabi *et al*., 2017). Finding a solution to the problem of the growth of *O. afrasiaticus* and the devastating consequences it is causing to the economic interests of date palm growers, has become extremely significant. The pest poses a serious threat to the crop as it can directly attack date fruits at all their developmental stages. This indirectly leads to uncharacteristic date fruits and decreasing yields which result in a huge economic loss. Additionally, complaints were received from the date palm growers that owing to the extensive use of the limited number of acaricides that is in turn due to the non-availability of a large variety of acaricides in the market, *O. afrasiaticus* has not been effectively controlled. This lack of variety of acaricides has contributed to the overuse of the recommended dose of acaricides by the date palm growers who do so as a precautionary measure to protect their crop from the date palm mite. As a result, a number of exported shipments were rejected and also an increase in the residual effects in date fruits and date fruit products has been observed. For example, El-Saeid and Al-Dosari (2010) tested the residual effects of four groups of pesticides from eight local markets of Riyadh, KSA, these insecticides include OCPs (*e.g.* Lindane and Dieldrin), OPPs (*e.g.* Dimethoate and Chlorpyrifos), pyrethroids (*e.g.* Deltamethrin and Cypermethrin), fungicides (*e.g.* Benomyl and Carbendazim), acaricides (*e.g.* Amitraz, Dicofol, Tourk and Abamectin) and herbicides (*e.g.* Glyphosate and Atrazine) in dates and seeds of three cultivars of date (Sukkari, Khalas, NaboutSeif). They found that the residual effects of all pyrethroids, herbicides, and fungicides tested in dates and their seeds were below the MRL (Maximum Residue Level) whereas the residual effects of OCPs, OPPs and acaricide residues in date fruit samples exceeded the MRL. They also found dimethoate residues in the date seeds. Since, date leaves, date fruit bunches and date seeds are sometimes used as animal feed that can unfortunately add further pesticide residues to the food chain.

There is not much published information on *O. afrasiaticus* control and Alhewairini and Al-Azzazy (2017a) documented that Huwa-San TR50 was very effective in killing the date palm mite. Moreover, Huwa-San TR50 has been successfully used as an acaricide in killing Varroa mite (*Varroa jacobsoni* Oudemans), two spotted spider mites (*Tetranychus urticae* Koch) with minimal effects on its associated predatory mite, (*Neosiulus cucumeris* Oudemans) (Alhewairini and Al-Azzazy, 2017b, 2018) and tomato russet mite, *Aculops lycopersici* (Massee) (Al-Azzazy and Alhewairini, 2018), without significant effects on honeybees (*Apis mellifera lamarckii*) and seven-spot ladybird beetles (*Coccinella septempunctata*) (Alhewairini, 2017). Huwa-San TR50 is substantially used as a disinfectant and was developed nearly 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).

Oxamyl belongs to carbamate group and has several trade names such as Vydate, DPX1410 and Thioxamyl (USEPA, 2004). It has been widely used as a broad spectrum pesticide to control insect pests in several countries including developed countries such as USA. Oxamyl does not persist in the environment and does not accumulate in the fat.

Polyether modified polysiloxane is an organosilicone based surfactant containing a unique blend of various polymers. It has several trade names such as Injo200, Consume and Maxx. It has been extensively used with agricultural pesticides as non-ionic wetter, spreader and penetrant.

According to the literature, oxamyl has not been tested or used in controlling *O. afrasiaticus* although it has acaricidal properties. Furthermore, the toxicity of oxamyl with Injo200 has also never been tested.

Present study aims at evaluating the toxicity of oxamyl against *O. afrasiaticus* and testing the potential of mixing Injo200 or Huwa-San TR50 with oxamyl to increase its effectiveness in controlling *O. afrasiaticus* under laboratory conditions.

MATERIALS AND METHODS

Solutions and experiment protocol

Oxamyl (Fymate 24% w/v oxamyl) and polyether modified polysiloxane (Injo200 24% w/v Polyether Modified polysiloxane) were obtained from Astra Company. The recommended application rates (4ml/1L for Fymate and 50ml/100L for Injo200) for direct spray mixture were only used in this study.

Huwa-San TR50 was provided by Alhayat Development Chemical Factory. The stock solution of Huwa-San TR50 (500,000ppm) was diluted with distilled water to give a concentration 1000ppm. The experiments

were performed during the month of May in 2017, under laboratory conditions.

Date fruit bunches were collected from untreated farms which were heavily infested by the date palm mite in Al-Badaya City, Al-Qassim, Saudi Arabia. Twenty-five fruit bunches were randomly selected for diagnosing the date palm mite infestation and between 4 and 6 spikelets were collected from each infested bunch of the date palm cultivar (Shakra). The collection of date fruits was arranged in a randomized complete block design. The collected date fruits were carefully placed into a clean plastic container and immediately transported to the laboratory (25±2ºC and 70% relative humidity) at the Department of Plant Production and Protection, College of Agriculture and Veterinary Medicine, Qassim University for bioassay.

Thereafter, a small scalpel and tong was used to detach collected date fruits from the spikelet to avoid touching the date fruits which might disturb its population. Date fruits were then divided into a Petri dish (14 cm) (a clean tissue paper was used as background), 10 date fruits/3 different treatments of quarter recommended dose (QRD), half recommended dose (HRD) and recommended dose (RD) of oxamyl and a control (distilled water) with 10 replicates. The recommended dose (RD) of Injo200 or 1000ppm of Huwa-San TR50 were mixed with QRD, HRD and RD of oxamyl to evaluate the potential of this mixture in increasing the toxicity of oxamyl against the date palm mite. The effects of RD of Injo200 and 1000ppm of Huwa-San TR50 on mortality and number of larvae hatching from eggs of the date palm mite under laboratory conditions were also tested. To determine pre-spray counts, the number of date palm mites was manually counted by direct observation. Three different concentrations of oxamyl (QRD, HRD and RD), the mixture of these concentrations with the RD of Injo200 or 1000ppm of Huwa-San TR50 and the control (distilled water) were then directly sprayed onto each Petri dish by using a small knapsack sprayer (1L). Afterwards, to determine post-spray counts, dead mites were counted daily up to one week after application.

On the other side, the eggs of the date palm mite were carefully collected by using a small wet brush so that the eggs viability was not disturbed. The collected eggs were then placed into a Petri dish (8 cm) on a green tissue paper to gain a clearer view of the eggs. Eggs were then exposed to the three different concentrations of oxamyl (listed above) including the mixture of RD of Injo200 or 1000ppm of Huwa-San TR50 and control (distilled water).

Statistical analysis

The equation of Henderson and Tilton (1955) was used to calculate the reduction percentages of the average population number of the date palm mite:

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

Where, n is the number of date palm mites, T is treated, Co is control and Tr is treatment.

The mortality percentages of the date palm mite were calculated manually by direct observation in each date fruit. Thereafter, Microsoft Excel Program was used to calculate the average obtained data and the percentage of the number of larvae hatching from eggs. Statistically, all variables of the obtained data were analyzed using one-way analysis of variance (ANOVA). Data for the mortality examines and number of larvae hatching from the eggs were plotted using Graphpad Prism version 7. The results are expressed as mean mortality percentage ± SEM for each treatment.

Table I.- Effect of QRD, HRD and RD of oxamyl only and mixing with the RD of Injo200 or 1000ppm of Huwa-San TR50 on *O. afrasiaticus* under laboratory conditions.

Treatments	No. of mites *O. afrasiaticus*								
	Oxamyl only			Oxamyl with Injo200			Oxamyl with 1000ppm of Huwa-San TR50		
	Avg. pre-spray count	Avg. pre-spray count*	Reduction** (%)	Avg. pre-spray count	Avg. pre-spray count*	Reduction** (%)	Avg. pre-spray count	Avg. post-spray count*	Reduction** (%)
Control	22.11	22.10	0.00 a	20.91	20.87	0.00 a	25.73	25.69	0.00 a
QRD	21.87	8.35	61.79 b	24.55	9.11	62.87 b	23.45	6.63	71.70 b
HRD	23.79	6.28	73.58 c	24.46	6.41	73.77 c	24.91	4.42	82.23 c
RD	20.98	3.58	82.90 d	22.70	3.78	83.32 d	26.56	2.02	92.37 d

*, 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 $P < 0.05$. QRD quarter recommended dose of oxamyl; HRD half recommended dose and RD recommended dose.

Table II.- Number of larvae hatching from eggs of the date palm mite treated with QRD, HRD and RD of oxamyl only and mixing with the RD of Injo200 or 1000ppm of Huwa-San TR50on *O. afrasiaticus* under laboratory conditions.

Tr	No. of eggs and larvae /fruit								
	Oxamyl only			Oxamyl with Injo200			Oxamyl with 1000ppm of Huwa-San TR50		
	Avg. No. of eggs pre-spray count	Avg. No. of larvae post-spray count*	Hatching** (%)	Avg. No. of eggs pre-spray count	Avg. No. of larvae post-spray count*	Hatching** (%)	Avg. No. of eggs pre-spray count	Avg. No. of larvae post-spray count*	Hatching** (%)
Control	29.41	29.39	0.00 a	41.72	41.69	0.00 a	26.79	26.77	0.00 a
QRD	28.44	27.65	97.20 b	29.37	27.82	94.70 b	28.44	15.38	54.01 b
HRD	30.33	28.71	94.62 b	40.89	38.17	93.32 b	38.34	22.22	57.93 b
RD	33.87	28.98	85.53 c	36.84	31.27	84.86 c	30.91	14.29	46.21 c

*, Average counts made one week post treatment; **, Hatching percentage calculated with Excel Microsoft program. Mean followed by the same letter in a column are not significantly different from each other at P > 0.05. QRD quarter recommended dose of oxamyl; HRD half recommended dose and RD recommended dose.

RESULTS

The toxicity of oxamyl on *O. afrasiaticus* was tested. This includes a new approach of mixing 1000ppm of Huwa-SanTR50 with all concentrations of oxamyl used. Oxamyl showed high effectiveness with 1000ppm of Huwa-SanTR50 more than itself and with the RD of Injo200. Its effectiveness was not only on the mortality but also on the number of larvae hatching from eggs of *O. afrasiaticus*. An immediate effect was seen after application of oxamyl with 1000ppm of Huwa-SanTR50 as the adult of *O. afrasiaticus* showed less mobility compared with both oxamyl only and with the RD of Injo200 as well as control (distilled water).

The mortality was increased with the three oxamyl concentrations (QRD, HRD and RD) and was significantly when co-applied with Huwa-SanTR50 compared with RD of Injo200 (Tables I, II). Statistically, oxamyl with 1000ppm of Huwa-SanTR50 had significant difference compared with oxamyl only and with Injo200 in both mortality and larvae hatching from eggs of *O. afrasiaticus* ($P < 0.5$ using F-test Graphpad prism 7) (Fig. 1).

The application of oxamyl with 1000ppm of Huwa-San TR50 showed an observed malformation in the cuticle of dead *O. afrasiaticus*as well as in the shell of non- hatched eggs compared with oxamyl only or with RD of Injo200. Clearly, Injo200 appears to have no significant effect on both mortality and number of larvae hatching from eggs of *O. afrasiaticus* when it is used alone compared with control (distilled water) whereas the mortality and number of larvae hatching from eggs percentages were 56.71 and 86.73%, receptively, after exposure to 1000ppm of Huwa-San TR50 (Table III). It is clear that mixing 1000ppm of Huwa-San TR50 with QRD, HRD and RD of oxamyl can significantly increase effectiveness of oxamyl in both the mortality and number of larvae hatching from eggs of *O. afrasiaticus* compared with control (distilled water).

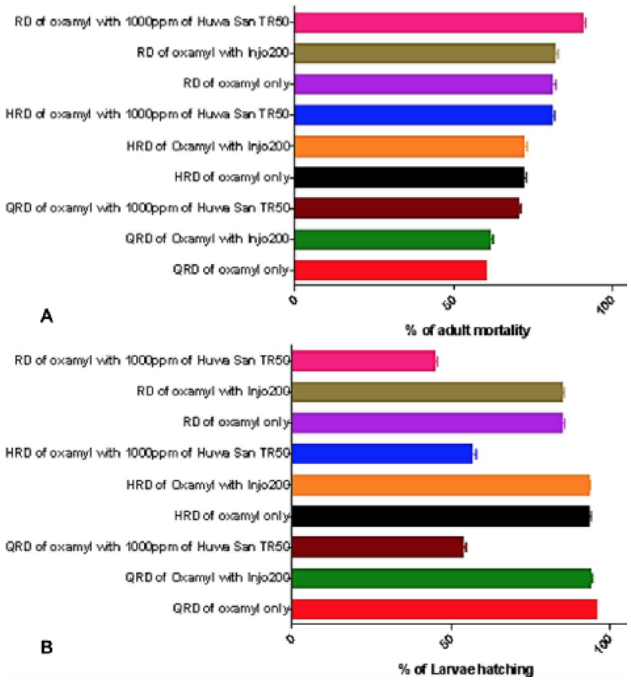

Fig. 1. The difference between the average effect of QRD, HRD and RD of oxamyl only and mixing with the recommended dose (RD) of Injo200 or 1000ppm of Huwa-San TR50 on the mortality (A) and larvae hatching from eggs of *O. afrasiaticus* (B) under laboratory conditions after one week of exposure, expressed as a percentage of the control mortality in distilled water. Each colored bar indicated different treatment that is significantly different compared with control (distilled water). Each point is the mean ± SEM of 10 replicates.

Table III.- Effects of the RD of Injo200 and 1000ppm of Huwa-San TR50 on mortality and number of larvae hatching from eggs of *O. afrasiaticus* under laboratory conditions.

Treatments	No. of mites O. afrasiaticus			No. of eggs and larvae /fruit		
	Avg. pre-spray count	Avg. post-spray count*	Reduction** (%)	Avg. No. of eggs pre-spray count	Avg. No. of larvae post-spray count *	Hatching*** (%)
Control	25.51	25.49	0.00 a	24.67	24.66	0.00 a
Injo200	27.15	27.14	0.00 a	38.89	38.87	0.00 a
1000ppm of Huwa-San TR50	30.17	16.11	56.71 b	32.47	29.16	86.73 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 not significantly different from each other at P > 0.05.

DISCUSSION

The date palm mite, *O. afrasiaticus*is considered the most economically important pest of date fruits, as it can cause a huge economic loss. Many health and environmental concerns have arisen regarding toxicity related problems and the growing incidences of mite resistance to available and recommended acaricides. Furthermore, mite infestation is not tolerated by date palm owners, hence they might exceed the recommended dose of acaricides for achieving the highest date palm mite mortality. Such an attitude is not only harmful to the treated date palm trees but also harmful to humans, domestic animals, natural enemies and could also accelerate resistance. This has greatly increased the need of several scientific researchers to find an effective and safe alternative strategy for date palm mite control. Therefore, the experiments in this study were designed to evaluate the toxicity of oxamyl on *O. afrasiaticus* and the potential of mixing RD of Injo200 or 1000 ppm of Huwa-San TR50 in increasing the efficacy of oxamyl.

The obtained results here showed that mixing 1000ppm of Huwa-San TR50 with oxamyl can cause a detectable malformation in the cuticle of dead *O. afrasiaticus* as well as in the shell of non- hatched eggs compared with oxamyl only or with the RD of Injo200. These findings were already reported in the cuticle of dead *O. afrasiaticus* after the application of certain concentrations of Huwa-San TR50 (Alhewairini and Al-Azzazy, 2017b).

Al-Dosary (2010) determined that the mortality percentages of *O. afrasiaticus* were 59.41, 60.78, 82.87, 86.48 and 95.83% for Final, Lord, Falcon, Baythroid and Albarq, respectively. In his results the mortality percentages of *O. afrasiaticus* eggs were 42.60, 49.60, 59.38, 69.46 and 82.87 % for Final, Lord, Baythroid, Albarq and Falcon, respectively. Moreover, he recorded that the egg-laying rate was decreased after application of these insecticides up to 21 days. This includes the mortality percentage that was increased with time up to 21 days. The results obtained here were consistent with his findings as the mortality percentages of oxamyl only and with the RD of Injo200 or 1000 ppm of Huwa-San TR50 were increased with time except oxamyl and 1000ppm of Huwa-San TR50 was more potent and showed a marked defect on the mobility of *O. afrasiaticus* immediately after the application.

The laboratory result of Alhewairini and Al-Azzazy (2017b) showed that the mortality percentage of adult *O. afrasiaticus* were 47.26, 58.83, 71.56, 86.79, 95.77, 97.02 and 99.69 after one week of exposure to 500, 1000, 2000, 3000, 4000, 5000 and 6000ppm of Huwa-San TR50, respectively.

They reported that > 97% mortality of the date palm mite was rapidly achieved after 48 h of applying 5000 and 6000ppm of Huwa-San TR50 whereas the mortality percentages of lower concentrations 500, 1000, 2000, 3000 and 4000ppm were increased with time. In comparison with the above mortality percentages, 82.90% was the maximum mortality percentage achieved by the RD of oxamyl, although, it has acaricidal properties. Alhewairini and Al-Azzazy (2017b) also highlighted that all mites exposed to Huwa-San TR50 did not show any recovery. Similarly, there was no recovery detected during this study with all oxamyl concentrations used. Therefore, this can emphasize the efficacy of oxamyl in controlling *O. afrasiaticus*.

On the other hand, there was insignificant effect on eggs exposed to oxamyl only or with the RD of Injo200 whereas oxamyl with 1000ppm of Huwa-San TR50 significantly resulted in reducing hatching percentage to 46.21% compared with control, although, 1000ppm of Huwa-San TR50 can reduce hatching percentage to 86.73% compared with control. A marked decrease (~50%) on eggs hatching was obtained after the application of oxamyl with 1000ppm of Huwa-San TR50. Furthermore, mixing 1000ppm of Huwa-San TR50with QRD and HRD of oxamyl can significantly reduce the hatching percentage to 54.01 and 57.93%, respectively. Therefore, this would

show how Huwa-San TR50 can significantly increase the efficacy of oxamyl.

The results obtained here were consistent with a previous study as Huwa-San TR50 has been proven to effectively kill adults as well as highly affecting egg viability (Alhewairini and Al-Azzazy, 2017b).

Higher mortality of *O. afrasiaticus* can be achieved by increasing the concentration of oxamyl but this is beyond the scope of this study and it may lead to serious health and environmental problems.

CONCLUSION

This study has provided a new acaricide that can be effectively used in controlling *O. afrasiaticus*. This includes the new approach of mixing Huwa-San TR50 with oxamyl which significantly increases the effectiveness of oxamyl against *O. afrasiaticus*. The addition of Huwa-San TR50 cannot only reduce the controlling costs but also can reduce residual effects of oxamyl. This would encourage us to investigate the potential of Huwa-San TR50 in increasing the effectiveness of other acaricides against *O. afrasiaticus*. It would also be valuable to evaluate egg-laying defects that may occur by the use of oxamyl alone or with 1000ppm of Huwa-San TR50 or Injo200, as it is still unknown whether they could affect it or not.

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Statement of conflict of interest

Authors declare that there is no conflict of interest.

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