

Determination of Biological Activities of Wood Samples Impregnated with Multi-Functional Compounds and Synergistic Effect Analysis Based on Fungal Resistance

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ABSTRACT

In this study, the biological resistance of Scotch pine (*Pinus sylvestris*) and beech (*Fagus orientalis*) wood samples which impregnated with individual and combinations of multi-functional compounds. Artificial antioxidant (A), GRAS compounds (G) and nano-SiO₂ (N) were chosen as multi-functional compounds. Firstly, the wood samples were impregnated single, binary and ternary combinations of impregnation solutions. Larvicidal resistance of pine samples was investigated against to *Hylotrupes bajulus*. Termiticidal resistance of all samples were determined using Subterranean termites *Reticulitermes*. Also, *Gloeophyllum trabeum* and *Poria placenta* were used for fungal resistance of beech and pine samples, respectively. Synergistic effects were calculated based on the fungal results. It was concluded that binary combinations of A+G and G+N and also the ternary combination (A+G+N) have a synergistic effect in binary variations for pine wood samples while A+G and A+N binary combinations show synergistic effect for beech wood samples. In addition, it is seen that the ternary combination also has a synergistic effect as in beech wood samples.

Keywords: Insecticidal, impregnation, fungal activity, synergistic effect, termiticidal.

1. INTRODUCTION

Wood material is considered as the only renewable raw material of humanity. Wood material has numerous advantages such as high resistance, easy processing, low energy consumption during processing, having different colors, low conductivity of sound, heat and electricity, informing danger before breaking, evaluating by converting into composite products, degree of acoustic properties, beautiful and natural appearance so on. Despite to the mentioned advantages, harmful organisms can cause the decay of organic wood and decrease its life. These organisms can be sorted as bacteria, fungi and insects. Therefore, especially non-durable wood species have always needed protection using different techniques¹.

Due to environmental concerns and disposal problems, the expectation of protecting wood using more environmentally and more naturally substances is increasing day by day. These concerns have led researchers to research more 'innocent' wood preservation technologies²⁻³. Also, another approach to protecting wood is to combine two or more active compounds to provide a synergistic formulation. The benefits of combining two or more biocides have been known for a long time, and their use increases the efficiency against various wood-destroying organisms by synergistic effect⁴.

The study is a first approach to investigate the influence of individual and combinations of commercially available formulations of selected artificial antioxidants, GRAS compounds and nano-oxides on the the

biological resistance of Scotch pine (*Pinus sylvestris*) and beech (*Fagus orientalis*) wood samples.

2. MATERIALS AND METHODS

2.1. Test Specimens

The test specimens cut from five, 45-50 years old trees for each specimens (*Pinus sylvestris* and *Fagus orientalis*) from Gümüşhane and Trabzon plantation located in north-eastern Turkey, respectively. Samples were taken from sapwood for both specimens and cut into 50 x 25 x 15 mm (longitudinal x radial x tangential) and conditioned 20 ± 2 °C and 65±3% relative humidity in the conditioning cabinet until their masses became stable.

2.2. Impregnation

Sodium ascorbate, sodium benzoate and SiO₂ were selected from artificial antioxidants, GRAS compounds and nano-oxides, respectively. Design of impregnation solutions were given in Table 1. The impregnation solutions were prepared at 1.0 % concentrations using distilled water and mixed on a shaker with 1000 rpm for 5 minutes.

Table 1. Design of impregnation solutions.

Code	Artificial antioxidant (%)	GRAS (%)	Nano-oxide (%)	Total (%)
1	1.0	-	-	1.0
2	-	1.0	-	1.0
3	-	-	1.0	1.0
4	1.0	1.0	-	2.0
5	1.0	-	1.0	2.0
6	-	1.0	1.0	2.0
7	1.0	1.0	1.0	3.0

The conditioned samples were first weighed, then subjected to impregnation in a medium-scale chamber. A vacuum of 635 mm Hg was applied for 15 minutes, followed by pressurization at 6 bar for 45 minutes. After treatment, the samples were removed from the solution, gently wiped to eliminate excess liquid from the wood surface, and reweighed with a precision of 0.01 g to calculate retention values. Untreated blocks served as controls. Retention for each concentration was determined using Equation 1.

$$R = \frac{(G \times C)}{V} \times 10 \text{ (kg/m}^3\text{)} \quad (1)$$

Where; Where: R represents the retention (kg/m³), G is the amount of treating solution absorbed by the samples, calculated by subtracting the initial mass of the samples from their mass after treatment (g), C denotes the concentration of the treating solution (%), and V is the volume of the samples (cm³).

2.3. Insect Test

Insect resistance of only pine samples was performed against to *Hylotrupes bajulus* in accordance with EN 47⁵. The larvae Category 2 were obtained from IVALLSA-CNR, Italy. Firstly, the healthy and white larva were chosen and weighed and the diameter of holes were determined based on mass of larvae (Table 2). Then one appreciate diameter hole (half the length of the wood) was drilled into each wood and the larvae were placed headfirst. After the larvae entering completely in hole, the hole was closed using cotton.

Table 2. Diameter of holes based on EN 47.⁵

Mass of larvae (mg)	Approximate diameter of holes (mm)
from 50 to 60	3.0
from 60 to 90	3.5
from 90 to 130	4.0
from 130 to 150	4.5

2.4. Termite Test

Termite test was utilized in accordance with EN 117 standard⁶. Worker, soldier and nymph termites were collected from termites' traps in Florence University. The termites have been identified through their morphologic characters. 250 workers and 3 soldiers and nymph were put on the glass contain white quartz sand (99.5% silica) and distilled water (4:1 volume ratio; 80 mL sand and 20 mL distilled water). Before the termites, approximately 0.5 g of the original host tree and a glass ring was placed on the sand-water mixture. Then the bottles contain termites were inoculated for 24 hours in testing chamber at 26±2 °C and 70±5 % relative humidity conditions. Then the wood samples were introduced the prepared glass bottle and exposed to termite attacks for 8 weeks. Three replicates per group were exposed to termites. End of the test period, and the number of live termite workers, soldiers and nymphs were counted in order to determine the survival rate (%). Visual examination of samples exposed termites given in Table 3.

Table 3. Visual examination of samples exposed termites.⁶

Visual examination	Degree of attack	Explanation
0	No Attack	No erosion
1	Attempted Attack	Superficial erosion
2	Slight Attack	Erosion of 1 mm in depth and/or single tunneling to a depth of up to 3 mm
3	Average Attack	Erosion of > 1 mm in depth and/or isolated tunneling to a depth of > 3 mm without enlarging to form cavities
4	Strong Attack	Erosion of > 1 mm to < 3 mm in depth and/or tunneling penetrating to a depth > 3 mm and enlarging to form a cavity in the body of the test specimen

2.5. Fungal Test

Fungal test was applied according to EN 113 standard⁷. *Gloeophyllum trabeum* and *Poria placenta* were used for fungal resistance of beech and pine samples, respectively. Firstly, 40 gr malt and 20 gr agar were mixed in 1 L distilled water and 60 mL of prepared liquor was poured into each glass bottle and then all bottles were sterilized at 121 °C for 30 minutes. After cooling the sterilized agar mediums, the mycelium was inoculated. The sterilized wood samples were put on the completely spread on agar medium and incubated for 16 weeks at 22±1 °C and 70±2 % relative humidity. After the fungal test, all samples were cleaned from mycelium and oven-dried at 103±2 °C for 24 hours. Dried samples were weighed and mass loss were calculated using the following Equation 2.

$$\text{Mass loss} = \frac{M_b - M_a}{M_b} \times 100 (\%) \quad (2)$$

Where, M_b is the oven-dried mass before the decay test, M_a is oven-dried mass after the decay test.

2.6. Synergistic Effect Calculation

The synergistic effect calculation was based on the results of the fungal rot test⁸. the synergistic effect between the two compounds and the three compounds is given in Equation 3 and 4, respectively.

$$E = X + Y - \frac{XY}{100} \quad (3)$$

$$E = X + Y + Z - \frac{XY + XZ + YZ}{100} + \frac{XYZ}{1000} \quad (4)$$

In the equations,

E: percentage of growth inhibition (%) expected from the mixture of chemicals studied,

X: Percentage of growth inhibition (%) of the first substance at concentration a,

Y: Percentage of growth inhibition (%) of the second substance at concentration a,

Z: Percent growth inhibition (%) of the third substance at concentration a.

When the observed response is greater than expected, the combination is synergistic; when it is less than expected, it is antagonistic⁸.

2.7. Statistical Analyses

The results were expressed as means ± standard deviations based on replicate measurements. Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS, version 23.0). Analysis

of variance (ANOVA) was employed to assess differences among groups, and Duncan's multiple range test was used to determine the significance of these differences.

3. RESULTS AND DISCUSSIONS

3.1. Retention

Retentions of impregnated samples (%) were given in Table 4.

Table 4. Retentions of impregnated samples (%).

Code	Retention (%)	
	Pine	Fagus
1	6.216 ± 0.421	5.514 ± 0.259
2	5.302 ± 0.719	5.483 ± 0.129
3	5.649 ± 0.637	5.435 ± 0.241
4	12.249 ± 0.782	11.730 ± 0.491
5	11.580 ± 0.582	11.404 ± 0.309
6	11.456 ± 0.685	11.179 ± 0.268
7	18.527 ± 1.839	18.210 ± 0.223

The retention rates of impregnated wood samples in both pine and beech species were generally similar; however, pine consistently exhibited slightly higher retention values. This may be attributed to the more porous structure of pine, which tends to absorb impregnation agents more effectively compared to beech. In the treatments coded as 1, 2, and 3, retention levels were relatively low, ranging approximately between 5% and 6% for both species. In contrast, treatments coded as 4, 5, and 6 demonstrated moderate retention levels, around 11% to 12%. The highest retention, approximately 18%, was observed in treatment code 7, which likely indicates the use of a higher concentration compound.

3.2. Insect test

Old house borer *H. bajulus* (Cerambycidae) is one of the most important insects that destroys wood structure especially coniferous trees such as pine, fir and spruce⁹. The larval development, which can last for 10 years or longer, is about two years under optimal conditions and is affected by parameters such as temperature, relative humidity, wood components so on¹⁰. *H. bajulus* is classified as 'economically important' and widely distributed all over the world, including Turkey¹¹.

Table 5. Insect test results.

Group no	Description	Insect Test Results	
		Number of larvae alive	Number of larvae dead
1	Antioxidant (A)	0-6	6-0
2	GRAS (G)	2-4	2-4
3	Nano-oxide (N)	0-1	0-1
4	A + G	0-0	6-6
5	A + N	0-0	6-6

6	G + N	0-0	6-6
7	A + G + N	0-0	6-6
8	Control	1-3	3-5

The rate of the chemicals studied to kill the larvae of the insect can be ranked from low to high as artificial antioxidant (A) < GRAS compound (G) < nano-oxide (N). In addition, all of the binary and ternary combinations of these chemicals killed all larvae during the test. In this study, it was concluded that nano-oxide alone or all binary and ternary combinations of the studied chemical groups were highly effective against *H. bajulus*.

The researchers investigated the natural resistance of indigenous and tropical wood species used extensively in the furniture industry against *H. bajulus* and *Anabium punctatum*. According to the results obtained, *Fagus orientalis*, *Cedrus libani* and *Populus tremula* and *Pinus sylvestris* were found to be the least resistant wood species against *H. bajulus*, while *Abies nordmanniana* wood samples were found to be the most resistant wood species. After the 12-week test period, larval mortality was lowest for *A. nordmanniana*. The largest sizes and weights of live larvae were measured in *P. sylvestris* wood¹¹.

Efficacy against insects depends on the toxicity of the active substance, the depth to which it can penetrate into the wood sample and its persistence or resistance to washing. *H. bajulus* digests 30 to 40% of hemicellulose/cellulose wood material, but their growth rate is limited by the amount of organic nitrogen available in the wood. The insects grow faster in areas where the nitrogen content is higher than in parts of the tree¹².

3.3. Termite Test

Termites are economically and ecologically important wood-destroying organisms worldwide, especially in the Mediterranean region, but also in tropics and subtropics around the world. It has been reported that *Reticulitermes* are the most abundant in Europe¹³. Termite resistance of impregnated pine and beech samples were given in Table 6 and Table 7, respectively.

Table 6. Termite resistance of impregnated pine samples.

Group no	Description	Visual assessment	Termite mortality (%)	Weight loss (%)
1	Antioxidant (A)	3.6 ^b (0.5)	53.4 ^{abc} (9.1)	12.5 ^d (1.4)
2	GRAS (G)	4.0 ^b (0.0)	50.6 ^{ab} (1.4)	11.1 ^{cd} (0.9)
3	Nano-oxide (N)	3.6 ^{ab} (0.5)	42.6 ^a (8.2)	13.0 ^d (3.6)
4	A + G	3.5 ^{ab} (0.7)	67.4 ^{bc} (10.6)	7.3 ^{ab} (2.0)
5	A + N	3.5 ^{ab} (0.7)	51.8 ^{ab} (9.8)	10.1 ^{bcd} (0.7)
6	G + N	3.5 ^{ab}	61 ^{bc}	8.1 ^{abc}

		(0.7)	(5.3)	(0.2)
7	A + G + N	2.6 ^a (0.5)	69.7 ^c (13.7)	5.1 ^a (2.4)
8	Control	4.0 ^b (0.0)	35 ^d (5.5)	18.0 ^e (7.3)

^aThe same exponential letters indicate that there is no statistically significant difference according to Duncan multiple comparison test (p>0.05)

^{*} Standard deviation value is given in parentheses.

Based on the visual evaluations of the yellow pine samples after the termite test, it is seen that there is no difference between the samples impregnated with GRAS and the control samples, the other single and binary combinations provide improvement at the same rate and the triple combination gives the best result.

Based on the termite mortality rates of yellow pine wood samples, the highest to lowest performance ranking in single impregnation applications can be made as nano-oxide, GRAS and antioxidant chemicals. In binary combinations, the performance ranking is A+G>G+N>A+N. The highest termite mortality rate was observed in the triple combination.

Based on the weight loss of yellow pine samples after termite test, the order of weight loss from highest to lowest in single impregnation applications was realized as nano-oxide, antioxidant and GRAS compound. In the binary combinations, the weight loss amounts can be made from the lowest to the highest as follows; A+N>G+N>A+G. The lowest weight loss was again seen in the ternary combination.

Table 7. Termite resistance of impregnated beech samples.

Grup no	Description	Visual assessment	Termite mortality (%)	Weight loss (%)
1	Antioxidant (A)	4.0 ^c (0.0)	65.8 ^d (5.0)	7.3 ^d (0.2)
2	GRAS (G)	4.0 ^c (0.0)	40.2 ^a (1.4)	7.7 ^d (0.6)
3	Nano-oxide (N)	4.0 ^c (0.0)	74.2 ^c (4.0)	7.3 ^d (0.4)
4	A + G	4.0 ^c (0.0)	52.4 ^b (0.0)	4.7 ^c (0.4)
5	A + N	0.0 ^a (0.0)	100 ^f (0.0)	1.6 ^{ab} (0.5)
6	G + N	0.6 ^b (0.5)	100 ^f (0.0)	2.1 ^b (1.2)
7	A + G + N	0.0 ^a (0.0)	100 ^f (0.0)	0.7 ^a (0.4)
8	Control	4.0 ^c (0.0)	60.6 ^c (4.9)	9.2 ^e (1.2)

^aThe same exponential letters indicate that there is no statistically significant difference according to Duncan multiple comparison test (p>0.05)

^{*} Standard deviation value is given in parentheses.

Based on the weight loss of beech samples after termite test, it is seen that there is not much difference between single impregnation applications. In binary combinations, the weight loss amounts can be listed as follows; A+G>G+N>A+N. The lowest weight loss was seen in the ternary combination.

Termite wood consumption is influenced by a wide range of complex factors. Among the most significant are the species and hardness of the wood, the presence of toxic compounds, feeding deterrents or inhibitors, the existence and extent of fungal colonization and decay, as well as the moisture content of both the wood and the surrounding soil ^{14,15}. Moreover, the correlation between wood density and termite attack resistance has been reported in previous studies ¹⁶⁻¹⁸.

Several studies have reported that the combination of different nanometals-such as silver nanoparticles with copper or zinc oxide-offers enhanced protection against termite attacks ^{19,20}.

Some researchers have reported a positive relationship between termite mortality and wood density in their study of termite. This result indicates that higher density species may be more resistant to damage by termite ¹⁸.

The factors affecting the wood consumption of termites are quite high and complex. The most important of these factors include tree species and hardness, the presence of toxic substances, inhibitors or deterrent substances, the presence or absence of fungi and the degree of fungal decay, the moisture content of wood and soil ^{15,17}.

3.4. Fungal Test

To date, fungi are the most common and most likely causes of structural degradation of wood materials ²¹. The weight loss values of pine and beech wood samples impregnated based on the selected chemicals and their combinations are shown in Figures 1 and 2, respectively.

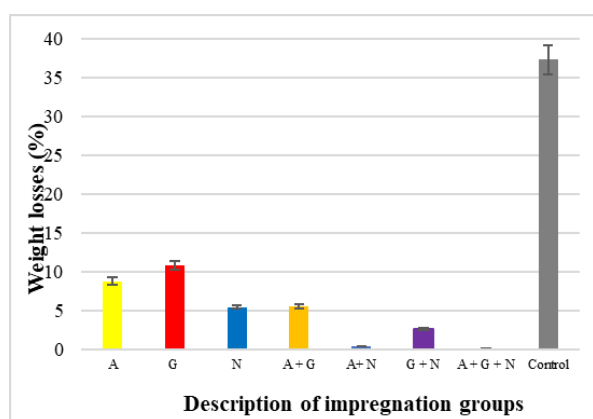


Figure 1. Weight loss values of pine wood samples.

Figure 1 summarizes the performance of pine wood samples subjected to fungal rot test according to EN 113 test standard against *Poria placenta* fungus. In single impregnation applications, it is seen that the weight losses are ranked from highest to lowest as GRAS> antioxidant> nano-oxide. In binary combinations, the

order of chemicals on the basis of weight loss was found as A+G>G+N>A+N. In binary combinations, the samples impregnated with the A+N combination suffered 4-10 times less weight loss than the samples impregnated with the other two combinations. The lowest weight loss was observed in the ternary combination.

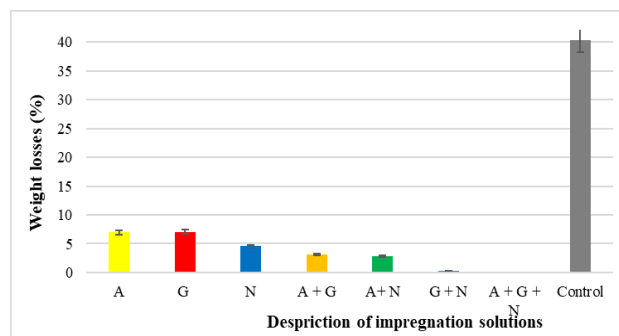


Figure 2. Weight loss values of beech wood samples.

When Figure 2 is examined, it is seen that when a ranking is made from the highest to the lowest in single impregnation applications of beech wood samples, it is seen that weight losses were realized as GRAS > antioxidant > nano-oxide, similar to yellow pine wood. In binary combinations, the weight losses were ranked from highest to lowest as A+G>A+N>G+N. In binary combinations, the samples impregnated with G+N combination lost 4-6 times less weight than the samples impregnated with the other two combinations. The lowest weight loss was again observed in the ternary combination.

Since the weight loss values in the control samples were above 20% in both wood species samples, the experiment was considered valid.

3.5. Calculation of Synergistic Effect

The synergistic effect calculation for pine and beech wood samples were given in Figures 3 and 4, respectively.

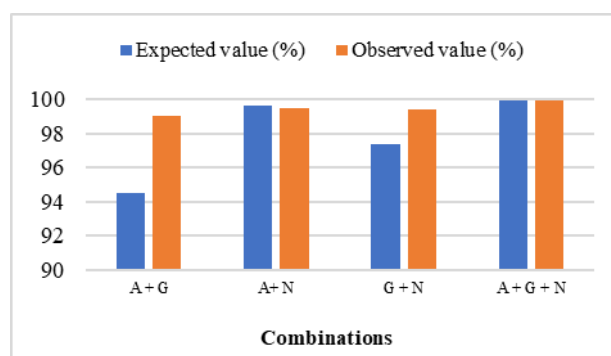


Figure 3. Calculation of synergistic effect in pine wood samples.

When the observed response is greater than expected, the combination is synergistic; when the observed

response is less than expected, the combination is antagonistic. Figure 3 shows that the binary combinations of A+G and G+N and also the ternary combination (A+G+N) have a synergistic effect in binary variations for pine wood samples.

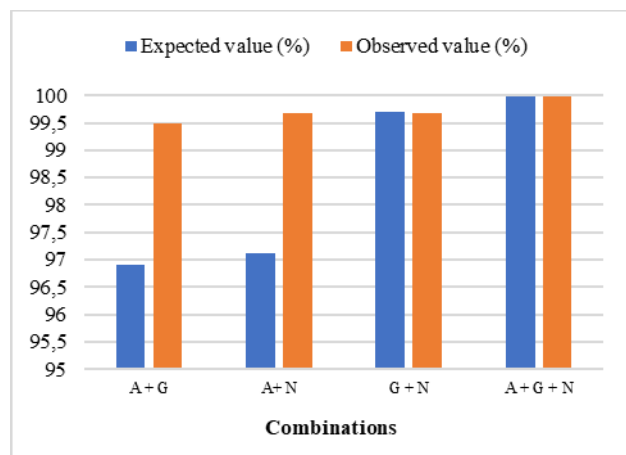


Figure 4. Calculation of synergistic effect in beech wood samples.

When Figure 4 is examined, it is seen that A+G and A+N binary combinations show synergistic effect for beech wood samples. In addition, it is seen that the triple combination also has a synergistic effect as in beech wood samples.

The synergistic effect of sodium benzoate, which was selected as the GRAS compound in this study, has been investigated in other studies²². The antimicrobial effects of sodium benzoate, sodium nitrite and potassium sorbate and their synergistic effects (sodium nitrite + sodium benzoate, sodium nitrite + potassium sorbate, sodium benzoate + potassium sorbate, sodium benzoate + potassium sorbate) on food spoilage bacteria and fungi (*Bacillus subtilis*, *Bacillus mycoides*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus flavus*, *Fusarium oxysporum*, *Candida albicans*, *Trichoderma harsianum* and *Penicillium italicum*). Sodium nitrite + sodium benzoate combination had a synergistic effect against 40% of the tested species (*E. coli*, *S. aureus*, *B. mucoides* and *C. albicans*) and sodium nitrite + potassium sorbate combination had a synergistic effect against 30% of the tested species (*B. mucoides*, *P. aeruginosa* and *E. coli*)²².

In a previous study it was emphasized that when antifungal drugs are limited, combining these chemicals with nanomaterials is promising for combating drug resistance. Nanomaterials have been reported to significantly increase their efficacy when combined with conventional antifungal drugs due to their highly specific surface area and special physical properties²³. Some researchers compared the polar antibacterial effects of silver nanoparticles with nystatin, myosin and ketoconazole and found that silver nanoparticles

enhanced the antifungal effect and significantly increased the percentage of fungal inhibition (90%-100%)²⁴.

In a study, it was investigated the antibacterial activity of magnesium oxide nanoparticles (MgO) alone or in combination with other antimicrobials (nisin and ZnO) against *Escherichia coli* and *Salmonella stanley*. The antibacterial activity of MgO solution increased with increasing MgO concentrations. Synergistic effect of MgO in combination with nisin was also observed. However, the addition of ZnO to MgO did not increase the antibacterial activity of MgO against both pathogens. These results suggest that MgO nanoparticles alone or in combination with nisin can be used as an effective antibacterial agent to enhance food safety²⁵.

Another researchers added chitosan, alginate and their half and half mixtures into the nano-ZnO solution to increase the shelf life of guava (*Psidium guajava* L) fruit and reported that the solution with chitosan added provided longer preservation than the other studied groups²⁶.

4. CONCLUSION

Considering similar studies and the results of this study, it can be said that the techniques of using chemicals or nano-oxides in combination will increase and become more widespread as the synergistic effect exists. In the wood preservation industry, this trend is expected to lead to the development of more effective and environmentally friendly treatment methods. Optimizing the combination of preservatives and nano-oxides can enhance the durability and resistance of wood products against biological degradation, while reducing the amount of harmful chemicals released into the environment. Future research should also focus on evaluating the long-term performance of these combined treatments under various climatic conditions to ensure their practical applicability in real-world scenarios.

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Conflict of Interest

The authors declare there are no conflicts.

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