

Effect of Some Pesticides and Wood Vinegar on Soil Nematodes in a Wheat Agro-Ecosystem

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ABSTRACT

In this study, the effects of some pesticides and wood vinegar on freeliving nematode trophic groups common in soil were investigated in a wheat field. Field experiments were conducted in 2014-2015 and 2015-2016 growing seasons in a winter wheat field with a randomized block design with four replicates in Muş Province, Turkey. The treatments were consisted of; 1) pesticide application (i.e., chemicals used for all wheat diseases, weeds and pests in the region), 2) 0.5, 1, 2, 3, 4 and 5% ml wood vinegar, which equates to pesticide application, and 3) untreated control In the field, 19 genera and one order of nematodes were found from five nematode trophic groups. In 2014-2015 and 2015-2016, the number of predator nematodes were 25 and 136, and the number of the most common plant parasitic nematodes were 3012 and 3657, respectively. From first to second growing season, the number of omnivore nematodes decreased, whereas other nematode groups increased. According to a simple correspondence analysis, it was determined that generally, there were significant connections between the treatments, and the measured properties and nematode trophic groups.

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Bazı Pestisitler ve Odun Sirkesinin Buğday Agro-Ekosistemindeki Toprak Nematodlarına Etkileri

ÖZET

Bu çalışmada, bazı pestisitlerin ve odun sirkesinin buğday tarlasında toprakta yaygın olarak serbest yaşayan trofik düzeyinde nematod gruplarına etkileri araştırılmıştır. Tarla denemeleri, Muş İlinde 2014-2015 ve 2015-2016 üretim sezonlarında kışlık buğday tarlasında tesadüf blokları deneme desenine göre dört tekerrürlü yapılmıştır. Muameleler; 1) pestisit uygulaması (bölgede buğdayda görülen hastalık, yabancı ot ve zararlılara karşı kullanılan kimyasal ilaçlar), 2) pestisit uygulamasına tekabül eden %0.5, 1, 2, 3, 4 ve 5 ml odun sirkesi uygulamaları ve 3) herhangi pestisit ve odun sirkesi uygulamasının yapılmadığı kontrol uygulaması şeklinde yapılmıştır. Deneme alanında beş trofik gruptan, 19 cins ve 1 takım düzeyinde nematod tespit edilmiştir. 2014-2015 ve 2015-2016'da avcı nematod sayısı sırasıyla 25 ve 136 olup, en sık rastlanan bitki parazit nematodların sayısı 3012 ve 3657'dır. 2014-2015 ve 2015-2016 yılları arasında omnivor nematod sayısı azalırken diğer nematod gruplarında artış gözlenmiştir. Basit uyum analizleri sonuçlarına göre, muameleler ile ölçülen özellikler ve trofik düzeyindeki nematod grupları arasında genel olarak anlamlı ilişkiler tespit edilmiştir.

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INTRODUCTION

Pesticides have been used extensively since the 1940s to protect agricultural production against the plant pathogens, pests and weeds (Tiryaki et al., 2010) and are considered to be a cause of environmental problems (Altıkat et al., 2009). In recent years, in plant protection applications, various alternative methods including natural products were investigated in response to the problems caused by the intensive use of synthetic pesticides (Erdoğan and Toros, 2005). Biopesticides emphasized as the derivatives of animals, plants, bacteria and various minerals (Yarsan and Cevik, 2007). Yin (2008), referring to Tsuzuki et al. (1989), reported that wood vinegar (WV) was used as natural organic pesticides (Yin, 2008). WV is obtain during the production of charcoal (Cai et al., 2012). Kim et al. (2008), referring to Jang (2004), reported that 80-90% of WV is composed of water, 10-20% of the organic compounds, with more than 200 organic acids but mostly acetic acid. WV is a good resource for organic agriculture and is widely used in agriculture and daily life in Japan (Mu et al., 2003). WV is moderately or slightly toxic so to non-target organisms in water and soil (Hagner, 2013). Koc (2019) stated that WV doses and its frequencies can have an effect on the numbers of nematode groups. Römbke et al. (2009) reported that application of the pesticides (fungicide benomyl, insecticide chlorpyrifos mixture and a new type of pesticide) to the grassland without mixing into the soil decreased the number of nematodes by 48% compared to the control. Daramola et al. (2015) reported that the population of nematodes in both sites where they applied carbofuran was significantly suppressed (P \leq 0.05). Chelinho et al. (2011), reported that in soil samples from agricultural areas exposed to carbofuran for 14 and 28 d the total number of soil nematodes and population of their families decreased, but there was no significant change in the trophic structure. The total number of nematodes, Shannon-Weaner index, species richness, sameness and number of species decreased in all contaminated microcosms. They found that WV induces development of reactive oxidative speciessensitive mutant nematodes, prolonging their life span and increasing body size (Cai et al., 2012). Despite the fact that pesticides are extensively studied for their possible effects on nematodes, serious studies are not conducted on the effects of wood vinegar as an organic pesticide for nematodes. The aim of this study was to determine the effects of pesticides (fungicide and herbicide) and WV on soil nematodes (according to trophic levels) in a wheat field.

MATERIAL and METHOD

Experimental Area

This study was conducted with winter wheat Krasunia Odeska in a field of Berce Alparslan Agricultural Administration (38°47'33", 41°32'46", 1276 m), 11.8 km from the center of Muş Province. The texture of the soil was clay with the proportion of clay, silt and sand was 63.3, 25.8 and 10.9%, respectively. Climate in Muş Province is terrestrial, winters are cold and snowy, and summers are generally short and cool (Anonymous, 2016a). Total average annual rainfall for the proceeding 10 years, and the total annual rainfall for the first (2014-2015) and second years (2015-2016) in the region were 741, 740 and 790 mm; with mean temperatures of 10.6, 11.6 and 11.5°C and mean RH of 60.8, 55.0 and 54.0%, respectively (Anonymous, 2016b).

Source of Wood Vinegar (WV), Pesticides and Fertilizer Materials

Broiler chicken farming waste was obtained from a company manufacturing biochar and WV products using a gasifier (Namlı et al., 2014). Fertilizer and pesticides were taken from Alparslan Agriculture Administration. In both seasons of 2014-2015 and 2015-2016, fertilizer for stand improvement (46% urea, 100 kg ha, Özbaşak, Öz Başak Tarım) and base fertilizers (N:P:K 20:20:0, 137 g ha) were used. In 2014-2015, pesticide Weed Killer D (herbicide, active ingredient: 2,4-D Acid Dimethylamin, 1000 ml ha, Koruma, Koruma Tarım) with Duett Super (fungicide, active ingredient: 84 g L Epoxiconazole and 250 g L Fenpropimorph, 1000 ml ha, Basf, BASF Agricultural Solutions Turkey) were applied to the plots and WV applied at 0.5, 1, 2, 3, 4 and 5% ml and water alone was applied as a control. Six liters of solution (pesticide, WV) was applied to testing area (each testing area was decided to be 100 m²). In 2015-2016, pesticide input (fungicide, active ingredient: 160 g L Prothioconazole + 300 g L Spiroxamine, EC, 1000 ml ha, Bayer, Bayer Crop Science), Harmony Platinum (herbicide, active ingredient: 37.5% Thifensulfuron methyl + 37.5% Tribenuron methyl, DF, 20 g ha, Bayer, Bayer Crop Science), Duett Super (fungicide, active ingredient: 84 g L Epoxiconazole and 250 g L Fenpropimorph, 1000 ml ha, Basf, BASF Agricultural Solutions Turkey), mixed with Attribut Super WG 20 (herbicide, active ingredient: 6.75% Propoxy carbazone-sodium + 4.5% Mesos ulfuron-methyl, WG, 200 g/ha, Bayer, Bayer Crop Science) and Biopower (1000 ml ha, Bayer, Bayer Crop Science) were applied. WV at 0.5, 1, 2, 3, 4- and 5%-ml concentrations and water alone as a control were applied.

Experimental Design, Pesticide and WV Applications

The experiment was set in a wheat field in a randomized block design with four replicates in both seasons. For each plot $(5 \times 5 \text{ m})$ there was a minimum distance of 2 m alleyway between plots and blocks. Pesticide were applied in the experiment (treatments) via backpack sprayer. Applied pesticides were routinely used ones for diseases, pests and weeds of wheat by Berce Alparslan Agriculture Administration according to their established application calendar. The following treatments were used in the study: 1) pesticide application; 2) 0.5% ml WV corresponding to pesticide application; 3) 1% ml WV corresponding to pesticide application; 4) 2% ml WV corresponding to

pesticide application; 5) 3% ml WV corresponding to pesticide application; 6) 4% ml WV corresponding to pesticide application; 7) 5% ml WV corresponding to pesticide application; and 8) control application (no pesticide and WV). Pesticide and WV application were done one times in 2014-2015 and four times in 2015-2016.

Soil Sampling

Soil samples were taken from eight different places in each plot (Yardim, 1996) with a 25 mm diameter sampling tube (10-30 cm deep). The soil samples were thoroughly mixed, placed in bags, and transferred to the laboratory momentarily, where they were stored at 4°C. In 2014-2015, the samples were taken before treatment application (19 May 2015), after treatment application (25 June 2015) and at the end of harvest (25 July 2015), and in 2015-2016, before treatment application (21 April 2016), after treatment application (25 June 2016) and at the end of harvest (9 August 2016).

Extraction, Identification and Counting of Nematodes

Nematodes were extracted from soil using a modified-Baermann funnel method (Baermann, 1917; Whitehead and Hemming, 1965; Southey, 1986). Nematode were counted under a light microscope at 10× magnification, and allocated to trophic groups described by Yeates (1971) and Yeates et al. (1993), and identified to genus.

Statistical analysis

Data were subjected to simple correspondence analysis in Minitab (Ver. 17) statistical package program (Winer et al., 1971).

RESULTS and DISCUSSION

The nematodes were found to belong to five trophic groups, 19 genera and one order (Table 1).

Effect of pesticides and WV on bacterivore nematodes

WV at 0.5-5% ml collected on 19 May 2015 exhibited similar responses to 3-4% ml samples collected on 25 July 2015, but WV at 2% ml showed no similarity or relationship with any other sample (Figure 1). On 21 April 2016, with WV at 5-0.5% ml and on 29 August 2016 with WV at 2% ml the relationship was with the highest number of nematodes. For WV at 1-3% ml and pesticide application the relationship with each other is similar, whereas the control is not related to any other sample (Figure 2). The total number of bacterivore nematodes in 2014-2015 (2834) was lower than those of 2015-2016 (3251). This increase can be attributed to the higher rainfall and weeds densities in 2015-2016. In 2014-2015, the number of bacterivore nematodes were the lowest for pesticide applications (262) and the highest for WV at 2% ml (493). In 2015-2016, again, the number of bacterivores were the lowest for WV at 3% ml (302) and the highest for WV at 0.5 ml WV (524) treatment. The lowest average number of bacterivores were observed in WV at 3% ml (292) and the highest was in WV at 5% ml (451) (Figure 3).

Table 1. Nematodes detected at the experimental area
Tablo 1. Deneme alanında tespit edilen nematodlar

Trophic group	Name (Genus)		
(Trofik grup)	(Adı (Cins))		
	Acrobeloides spp.		
e e	Acrobeles spp.		
tod	Achromadora spp.		
nat n <i>a</i> i	Cervidellus spp.		
nen 1er	Cephalobus spp.		
er 1. 10	<i>Eucephalobus</i> spp.		
701 1770	<i>Monhystera</i> spp.		
eri er	Plectus spp.		
ak	Prismatolaimus spp.		
(B_{Ba})	<i>Wilsonama</i> spp.		
Fungivore nematode (<i>Fungivor</i> <i>nematod</i>)	<i>Aphelenchoides</i> spp. <i>Aphelenchus</i> spp.		
Omnivore nematode (<i>Omnivor</i> <i>nematod</i>)	Dorylaimida (order)		
Predator nematode (<i>Avci</i> <i>nematod</i>)	<i>Mononchus</i> spp.		
itic zit	Ditylenchus spp.		
ura	<i>Merlinius</i> spp.		
par Da (par Da)	Pratylenchus spp.		
t 1 ato <i>ti</i> <i>atc</i>	<i>Tylenchus</i> spp.		
an Sitk Sitk	Tylenchorhynchus spp.		
ре Пре Пре	<i>Trophorus</i> spp.		

Compared to the control, bacterivore nematode populations increased in parallel to the climate and plant phenology, which was consistent with the study of Chelinho et al. (2011). However, in the second year of the study Koc (2019), bacterivore nematodes were found decreasing. Also, there are other previous studies reporting similar results. For instance, bacterivore nematode populations were the lowest in the broad-spectrum pesticide (fungicide and herbicide) treated areas (Yardim and Edwards, 1998; Johnson et al., 1981). Nematodes numbers in the soil (treated with organic phosphate or carbamate) were also reduced, and likewise for some pesticides applied to the grassland without mixing into the soil reducing nematode numbers by 48% (Römbke et al., 2009). Additionally, Soltani et al. (2012) reported that the total number of nematodes, species richness and number of species fell in all contaminated microcosm where permethrin applied.

Effect of pesticides and WV on plant parasitic nematodes

WV at 5% ml was most related to nematode number for the sample collected on 19 May 2015, whereas, on 25 June 2015 pesticide application and WV at 1 and 2% ml sustained the strongest relationship (Figure 4).



Figure 1. Simple correspondence analysis for the relationship between treatments and sampling date in 2014-2015 for the number of bacterivore nematodes

Şekil 1. Bakterivor nematod sayısı bakımından 2014-2015 yılı muamele ve örneklem tarihleri arasındaki ilişkiler için basit uyum analizi



Figure 2. Simple correspondence analysis for the relationship between treatments and sampling date in 2015-2016 for the number of bacterivore nematodes

Şekil 2. Bakterivor nematod sayısı bakımından 2015-2016 yılı muamele ve örneklem tarihleri arasındaki ilişkiler için basit uyum analizi



Figure 3. Based on the treatments, comparison of the total number of bacterivore nematodes between 2014-2015 and 2015-2016

Şekil 3. Muamelelere göre 2014-2015 ve 2015-2016 yılları arasındaki toplam bakterivor nematod sayısının karşılaştırılması



Figure 4. Simple correspondence analysis for the relationship between treatments and sampling date in 2014-2015 for the number of plant parasitic nematodes

Şekil 4. Bitki paraziti nematod sayısı bakımından 2014-2015 yılı muamele ve örneklem tarihleri arasındaki ilişkiler için basit uyum analizi

This can be explained with an increase in the number of plant parasitic nematodes. On 21 April 2016 with WV at 0.5% ml experiment exhibited the highest relationship with nematode number. Whereas on 25 June 2016, the control and on 9 August 2016, 3% ml WV, 1 and 2% ml WV applications were thought to be related or similar with the same treatment-like responses (Figure 5).

In 2014-2015 season, minimum number of plant parasitic nematodes were counted at 0.5% ml WV application (277 nematodes) and the maximum

number of plant parasitic nematodes were found at 1% ml WV application (527 nematodes). In 2015-2016 season, on the other hand, minimum number of plant parasitic nematodes were detected at 1% ml WV (326 nematodes) while the maximum number of nematodes were at 4% ml WV application (600 nematodes). As the average of this two seasons, maximum and minimum number of nematodes were found in 2% ml WV (486 nematodes) and Pesticides (345 nematodes) applications, respectively (Figure 6).

It is thought that this situation can be related by

changes in the density of weeds together with the extract applications. Compared to 2014-2015 (3012), in 2015-2016 (3657) the number of plant parasitic nematodes was higher. It is assumed that population of plant parasitic nematodes was affected by rainfall and weeds densities. In general, fluctuations in the number of plant parasitic nematodes are attributed to plant growth and development. These findings were similar to those of Chelinho et al. (2011) and Koc (2019), however some different results were also reported in different studies. For example; plant parasitic nematode populations were more common in the full spectrum pesticide treatment and insecticide treated than in control (Yardim and Edwards, 1998); some of the pesticides they applied to the soil decreased root-knot nematodes in the soil (Johnson et al., 1981); some of the pesticides they applied to the soil decreased the number of nematodes by 48% (Römbke et al., 2009); thus, pesticide applied to soil affects nematodes (Soltani et al., 2012).



Figure 5. Simple correspondence analysis for the relationship between the application and sampling in 2015-2016 for the number of plant parasitic nematodes





Figure 6. According to the treatments, comparison of the total number of plant parasitic nematodes between 2014-2015 and 2015-2016



Effect of pesticides and WV on fungivore nematodes

Samples collected on May 19th (2015) may be related to a continuous reduction in pesticide application. Further, analysis of the samples collected on July 25th (2015) indicated that, responses of 4%, 0.5% WV and control to pesticide application were similar. 3% mL WV applied on samples (collected on June 25th, 2015) observed results relatively increased and decreased before and after the application of WV (Figure 7). The results from this study coincide with Chelinho et al. (2011) however, previous researchers also reported that pesticide application can cause negative impacts on fungivore nematodes (Yardim and Edwards, 1998), and typically reduces the number of nematodes (Johnson et al., 1981; Römbke et al., 2009; Soltani et al., 2012). Sampling performed on June 25th (2016) related with of 1% WV. Similarities among 0.5%, 5% and 3% WV can be related to the responses of these WV to treatments. Similarly, 4% and 2% mL WV applications may be related their responses to pesticide treatment. Sampling on August 9th (2016) and April 21st (2016) were performed before pesticide and WV application and at the end of harvesting season; therefore, these samples were excluded from all the agricultural treatments including pesticide application (Figure 8).



Figure 7. Simple correspondence analysis for the relationship between treatments and sampling date in 2014-2015 for the number of fungivore nematodes

Şekil 7. Fungivor nematod sayısı bakımından 2014-2015 yılı muamele ve örneklem tarihleri arasındaki ilişkiler için basit uyum analizi



Figure 8. Simple correspondence analysis for the relationship between treatments and sampling date in 2015-2016 for the number of fungivore nematodes

Şekil 8. Fungivor nematod sayısı bakımından 2015-2016 yılı muamele ve örneklem tarihleri arasındaki ilişkiler için basit uyum analizi

Fungivore nematodes were determined as 201 (for the minimum pesticide application) and 335 (for the maximum WV application, 5% mL) for 2014 and 2015, respectively. Moreover, fungivore nematodes were determined as 204 (for the minimum WV application, 3% mL) and 394 (for the maximum pesticide application) for 2015 and 2016, respectively. Figure 9 shows the average fungivore nematode numbers, for the application of minimum (3% mL) and maximum (5% mL) WV, as 216 and 300, respectively.

In 2015-2016, the total number of fungivore nematodes increased from 2049 to 2198 compared to 2014-2015. This may be related to increase in the amount of precipitation and weed densities in the application field. Fungivore nematodes increased more in pesticide application in 2015-2016 during the WV treatments (Figure 10). This can be explained with negative effects of pesticides on omnivorous nematodes (Figure 13). Relatively different results were obtained compared to previous studies (including Johnson et al., 2009; Yardim and Edwards, 1998; Soltani et al., 2012).



Figure 9. According to the treatments, comparison of the total number of fungivore nematodes between 2014-2015 and 2015-2016

Şekil 9. Muamelelere göre 2014-2015 ve 2015-2016 yılları arasındaki toplam fungivor nematod sayısının karşılaştırılması

Effect of pesticides and WV on omnivore nematodes

Samples collected on May 19th (2015) were related to 0.5% mL WV and the highest number of nematodes observed. Samples collected on July 25th (2015), on the other hand, were related to 3% mL WV and relatively lower number of nematodes detected. 1% and 2% mL of WV showed similar responses to the pesticide and WV application. It is assumed that 5% WV and pesticide are related to each other due to similar responses to treatments (Figure 10).

Sampling analysis (for the specimen collected on April 21st 2016) exhibited an increase in the number of observed nematodes. This was related the application of 0.5% WV. Additionally, similar responses of 1%, 5% mL and control group to treatments may be related to interaction between these groups (Figure 11). Omnivore nematodes were determined as 18 (for the minimum WV application, 2% mL) and 35 (for the maximum pesticide application) for 2014 and 2015, respectively. Further, omnivore nematodes were determined as 20 (for the minimum control) and 32 (for

the maximum 1% mL WV application) in 2015-2016, respectively.

Figure 12 shows the average omnivore nematode number, for the application of minimum (control) and maximum (1% mL) WV, as 20 and 33, respectively. In 2015-2016, the total number of fungivore nematodes decreased from 211 to 202 compared to 2014-2015. This decrease of omnivores may be related to pesticide and WV applications in 2015-2016. These findings were found to be consistent with some previous studies (such as Johnson et al., 1981; Yardim and Edwards, 1998; Römbke et al., 2009; Soltani et al., 2012).

Effect of pesticides and WV on predator nematodes

According to the sampling analysis (with samples collected on July 25th, 2015), pesticide application can be related to the highest number of nematodes. Also, negative effects of pesticide and WV application on predator nematodes were found (Figure 13). Sampling performed on April 21st (2016) and August 9th (2016) were related to 5% mL WV and pesticide application,

respectively. Similar responses among 2% and 0.5% WV were related to a functional interaction between 2% and 0.5% WVs. Additionally, 3% WV and Control group were associated to each other due to their similar responses towards the applied treatments (Figure 14).

Predator nematodes were determined as 1 (for the minimum WV application, 3% mL) and 5 (for the maximum pesticide application) in 2014-2015, respectively. Moreover, predator nematodes were

determined as 6 (for the minimum WV application, 1% mL) and 32 (for the maximum WV application, 4% mL) in 2015-2016, respectively. The average predator nematode number, for the application of minimum (1% mL) and maximum (4% mL) WV, were determined as 4 and 17, respectively. The total number of predator nematodes increased from 25 in 2015-2016, to 136 in 2014-2015. This may be because of a numerical increase in other nematode groups except for omnivores (Table 2).



Figure 10. Simple correspondence analysis for the relationship between treatments and sampling date in 2014-2015 for the number of omnivore nematodes

Şekil 10. Omnivor nematod sayısı bakımından 2014-2015 yılı muamele ve örneklem tarihleri arasındaki ilişkiler için basit uyum analizi



Figure 11. Simple correspondence analysis for the relationship between the application and sampling in 2015-2016 for the number of omnivore nematodes

Şekil 11. Omnivor nematod sayısı bakımından 2015-2016 yılı muamele ve örneklem tarihleri arasındaki ilişkiler için basit uyum analizi



Figure 12. According to the treatments, comparison of the total number of omnivore nematodes between 2014-2015 and 2015-2016



In contrast to these findings, some previous studies reported that the number of predator nematodes can be adversely affected in the full spectrum pesticide treatments (Yardim and Edwards, 1998; Johnson et al., 1981; Römbke et al., 2009; Soltani et al., 2012).

In both 2014-2015 and 2015-2016 seasons, the lowest number of predator nematodes (25/136) and the greatest number of parasitic nematodes (3012/3657)

were determined, respectively. A decrease in the number of omnivorous nematodes in 2014-2015, and an increase in other nematodes in 2015-2016 were observed (Table 2). According to the results, significant relations between WV, pesticide treatments, and nematode groups were determined. Additionally, it is recommended that further studies are needed for biopesticide potential of wood vinegar on nematodes.



Figure 13. Simple correspondence analysis for the relationship between treatments and sampling date in 2014-2015 for the number of predator nematodes

Şekil 13. Avcı nematod sayısı bakımından 2014-2015 yılı muamele ve örneklem tarihleri arasındaki ilişkiler için basit uyum analizi









Figure 15. According to the treatments, comparison of the total number of predator nematodes between 2014-2015 and 2015-2016

Şekil 15. Muamelelere göre 2014-2015 ve 2015-2016 yılları arasındaki toplam avcı nematod sayısının karşılaştırılması

Table 2. Total number of nematodes in trophic groups in 2014-2015 and 2015-2016 Tablo 2. 2014-2015 ve 2015-2016 yılları trofik gruplarına göre toplam nematode sayısı

1abio 2. 2014 2015 ve 2015 2016 yman tronk grupianna gore topiani nematoue sayisi					
Trophic group (<i>Trofik grup</i>)	Years (<i>Yıllar</i>)		Total (<i>Toplam</i>)		
	2014 - 2015	2015-2016			
Bacterivore nematode (<i>Bakterivor nematod</i>)	2834	3251	6085		
Plant parasitic nematode (<i>Bitki paraziti nematod</i>)	3012	3657	6669		
Fungivore nematode (<i>Fungivor nematod</i>)	2049	2198	4247		
Omnivore nematode (<i>Omnivor nematod</i>)	211	202	413		
Predator nematode (Avci nematod)	25	136	161		
Total (Toplam)	8131	9444	17575		

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Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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