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Screening for pesticide residues in soil and crop samples in Egypt

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Abstract

Pesticide application is an important issue in intensive agricultural production to achieve self-sufficiency in food production. This study aimed to the determination of pesticide residues from soil samples cultivated with different crops. Forty soil samples from four governorates, ten samples each (Kafr El-Sheikh, El-Beheira, Giza, and Menoufia) were collected for pesticides multi-residual analysis. Also, fifteen vegetable samples were collected from Giza governorate and analyzed for the presence of pesticide residues. Obtained data revealed that soil samples of Giza governorate recorded the highest level of pesticide residues contamination with 70% positive samples followed by Kafr El-Sheikh and Menoufia governorates with 60% positive samples and finally El-Beheira governorate recorded the lowest level of pesticide residues contamination with 30% positive samples. Regarding the levels of pesticide residues in vegetables and crops, about 53.3 % of tested samples (onion, sweet potato, molokhia, cabbage, beet, okra, eggplant, and lettuce) were free from pesticide residues. Meanwhile, about 46.7 % of tested samples (wheat, rocca, green dill, tomato, radish, pepper, and green parsley) showed pesticide residues with different types and concentrations. Propiconazole was the highest residue recorded in green dill with 3.8 mg/kg followed by difenoconazole with 3.22 mg/Kg present in green parsley.

Keywords: Pesticide residues, contamination, determination, vegetable crops.

1. Introduction

The use of pesticides plays an important role in increasing the agricultural productivity of different crops, but the excessive use of pesticides negatively affects the environment in general and humans in particular. The negative effect of pesticides on humans extends from mild symptoms such as headache and dizziness, up to cancer, fertility diseases, and endocrine problems. The negative impact of pesticides on children is increasing [1, 2]. Pesticides are used to combat insects that attack vegetable crops, especially vegetables and fruits, to reduce losses of these crops. However, the excessive use of these pesticides leads to the presence of their residues in the soil as well as in foods at levels higher than permissible, which leads to stopping their export to foreign markets and thus leads to great economic losses [3].

There are many routes of exposure to contamination with pesticides such as air and water, but food is considered the main route of contamination by pesticides. The World Health Organization reported that fruits and vegetables are the most consumed food groups. Moreover, because vegetables and fruits are consumed raw or semi-processed, it is expected to contain high amounts of pesticide residues [4-6]. The consumption of fruits and vegetables contaminated with pesticide residues leads to adverse health effects due to its toxic and bioaccumulation properties. Cancers, birth defects, neurological disorders, and reproductive effects are the major health impacts associated with pesticide consumption [7].

Pesticide residues, which contaminate soil can be uptake by the plant and contaminates the crops which are considered a major concern with a respect to their safety for human and animal consumption [8]. Many reports showed that significant amounts of pesticide residues in soils can be taken up by plant roots and transferred to edible parts such as leaves and/or fruits

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[8-10]. Roots and tuber crops may be the most susceptible crops to contamination by pesticide residues because the pesticide residues absorbed from soils are mostly retained in the roots of the plants [11, 12]. The pesticide uptake mechanisms from soils may be via the plant water uptake system (*i.e.* via roots) or diffusion across their surface cuticle [13, 14]. The present work aimed to a determination of pesticide residues in soil samples as well as vegetable samples and assessing the persistent pesticide residues in each governorate.

2. Materials and Methods

2.1. Sampling

A total of forty soil samples were collected from four Egyptian governorates (Kafr El-Sheikh, El-Beheira, Giza, and Menoufia). Each sample weighed 1 kilogram of soil. In one farm, a final sample of soil was drawn from well-mixed samples of soil collected at different plotting, then placed in an opaque plastic bag, and taken for laboratory analysis. A soil auger was used to get the soil samples from a depth of 30 cm. All soil samples were placed in an ice-box and delivered to the laboratory within 24 h. The samples were stored in a laboratory refrigerator at a temperature of 5^{o} C and analyzed using gas chromatography. Also, fifteen vegetable samples (wheat, onion, sweet potato, molokhia, rocca, cabbage, green dill, beet, tomato, radish, okra, pepper, eggplant, lettuce, and green parsley) were collected from Giza governorate and analyzed for determination of pesticide residues using GC-MS/MS system.

2.2. Sample Extraction and Clean-Up

The extraction and clean-up method used was based on QuEChERS (quick easy cheap effective rugged and safe) sample preparation method for pesticides [15]. An aliquot of 15 g of homogenized sample was placed in a 50 mL centrifuge, and 15 mL of acetonitrile was added. The mixture was vortexed for one minute, followed by adding 4 g of magnesium sulphate and 1 g of sodium chloride. The sample was centrifuged, and the supernatant was removed for clean-up. The clean-up was carried out by transferring the supernatant into another tube containing 25 mg of primary and secondary amine (PSA), 12.5 mg of graphite carbon black (GCB), and 75 mg of $MgSO_4$. After this clean-up procedure, the final dried extract was dissolved in 0.5 ml acetonitrile and subjected to gas chromatography-mass spectrometry (GC-MS) analysis.

2.3. GC-MS/MS system and analysis conditions

Pesticides analysis was done using gas chromatography model 6890 with a mass selective detector model 5975 (Agilent Technologies, Little Falls, DE, USA) equipped with a Gerstel Dual rail MPS-2 Prepstation with DPX option (Linthicum, MD, USA). The Rtx-5 column (5% diphenyl/ 95% dimethyl polysiloxane, 30 m × 0.25 mm id, 0.25 µm film thickness, Restek crop., Bellefonte, PA, USA) was used for the separation of pesticides. Detected pesticides in this study were screened for 407 types of pesticides.

3. Results and Discussion

The presence of pesticide residues in the vegetable and soil samples collected from different Egyptian regions was determined by GC-MS/MS and the qualitative and quantitative analyses of pesticide residues were evaluated.

3.1. Pesticide residues in vegetable samples

The detection of pesticide residues in vegetable commodities, to optimally evaluating vegetables' quality and mitigating potential risks to human health, is a predominant aim of pesticide research. Most of the health effects of pesticides in humans are due to direct exposure either occupational, for agricultural workers who apply pesticides, or by self-poisoning. Fifteen samples of vegetables and crops were collected from Giza governorate and analyzed for qualitative (**Figure 1**) and quantitative analyses (**Table 1**) of pesticide residues. The obtained data revealed that about 53.3 % of tested samples (onion, sweet potato, molokhia, cabbage, beet, okra, eggplant, and lettuce) were free from pesticide residues. Meanwhile, about 46.7 % of tested samples (wheat, rocca, green dill, tomato, radish, pepper, and green parsley) showed pesticide residues with different types and concentrations. Propiconazole was the highest residue recorded in green dill with 3.8 mg/kg followed by difenoconazole with 3.22 mg/Kg present in green parsley, which attributes to the heavy application of propiconazole and difenoconazole during the period between the growing and harvest time of the plant.

Carbendazim is a systemic fungicide, which applied for controlling fungal diseases in vegetable crops. Carbendazim was detected only in the wheat sample as 0.02 mg/Kg that was lower than those detected by Lewandowska and Walorczyk [16] as 0.19 mg/Kg. Also, the carbendazim level was below maximum permissible limits (MPL) in cereals that vary among different countries in the range of 0.1-1.0 mg/kg [17].

Propiconazole and difenoconazole as triazole group fungicides were below limit of detection in rocca samples. Meanwhile, levels of propiconazole in green dill samples (3.8 mg/kg), as well as, difenoconazole in green parsley samples (3.22 mg/kg) were higher than MPL (0.05 mg/kg) according to USDA [18]. In contrast, levels of propiconazole in green parsley (0.01 mg/kg) and difenoconazole in green dill samples (0.01 mg/kg) were lower than MPL. Propiconazole and difenoconazole are metabolized by the different plants and may undergo oxidation, reduction, hydrolysis, or degradation into other compounds [19].

Lambda-cyhalothrin is an insecticide used to control a wide spectrum of insect pests in vegetables [20]. Residues of lambda-cyhalothrin were found in rocca and green dill as 0.031 and 0.01 mg/kg, respectively. All detected levels of lambda-cyhalothrin were lower than that recorded by another Egyptian study [21]. Only, level of lambda-cyhalothrin in rocca sample was higher than MPL (0.02 mg/kg) according to USDA [18].

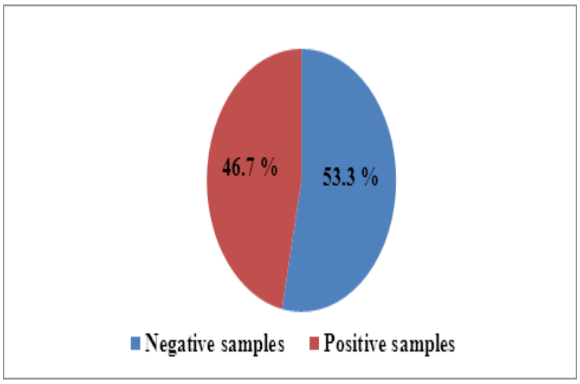

Fig. 1. Positive and negative percentage of pesticide residues incidence in vegetable samples collected from Giza governorate.

Atrazine is one of the most widely used herbicides for controlling weeds and grasses [22]. Atrazine was detected only in green parsley samples with a low concentration of 0.01 mg/kg and was below the limit of detection in rocca samples. Meanwhile, methamidophos as an organophosphorus pesticide was detected only in rocca samples with a low concentration of 0.03 mg/kg. These results are in disagreement with Yu et al. [23] who reported that methamidophos was detected in many vegetables such as cabbage, cucumber, tomato, onion, radish, and brinjal.

TABLE 1. Pesticide concentrations in the analyzed vegetable samples collected from Giza governorate

Cultivated crop	Detected pesticide	Concentration (mg/Kg)
Wheat	Carbendazim	0.02
Onion	Not Detected	
Sweet potato	Not Detected	
Molokhia	Not detected	
Rocca	Propiconazole	<LOQ
	Difenoconazole	<LOQ
	Lambda-Cyhalothrin	0.031
	Atrazine	<LOQ
	Methamidophos	0.03
Cabbage	Not detected	
Green dill	Piperonyl butoxide	0.01
	Difenoconazole	0.01
	Diazinon	0.01
	Cypermethrin	0.07
	Lambda-Cyhalothrin	0.01
	Propiconazole	3.8
	Profenofos	0.03
	Pendimethalin	0.04
	Malathion	0.07
	Chlorpyrifos	0.14
Beet	Not detected	
Tomato	Chlorfenapyr	0.01
	Cypermethrin	0.01
	Propargite	0.06
	Chlorpyrifos	0.01
Radish	Cypermethrin	0.01
Okra	Not detected	
Pepper	Chlorpropham	0.01
Eggplant	Not detected	
Lettuce	Not detected	
Green parsley	Propiconazole	0.01
	Malathion	0.01
	Cypermethrin	<LOQ
	Chlorothalonil	1.3
	Atrazine	0.01
	Dimethomorph	0.06
	Difenoconazole	3.22
	Chlorpyrifos	1.74
	Azoxystrobin	0.15

LOQ: Limit of quantification.

Piperonyl butoxide, diazinon, profenofos, and pendimethalin were found only in green dill samples as 0.01, 0.01, 0.03, and 0.04 mg/kg, respectively. The levels of pesticides in the current study were similar to that recorded by Bempah et al. [24] who reported that levels of diazinon and profenofos in the different vegetables ranged from 0.01 to 0.04 and from 0.003 to 0.055 mg/kg, respectively. The high levels of pendimethalin maybe return to its persistence in plant and soil as reported by Chopra et al. [25] who reported that residues of pendimethalin persisted up to 90 days. Gad-Alla et al. [26] reported that profenofos was detected in 12 samples in 11 different commodities from 2 countries and the highest value was 1.09 mg/kg which was recorded in strawberry.

Insecticide such as cypermethrin was detected in green dill (0.07 mg/kg) and tomato (0.01 mg/kg) and was below MRL (0.5 mg/kg) as reported by

FAO/WHO [27]. Meanwhile level of cypermethrin in green parsley was below the limit of detection. Al-Antary et al. [28] revealed that cypermethrin was found in 43 samples in 22 different commodities from 6 countries and the highest value recorded in parsley was 4.6 mg/kg. Malathion (organophosphate insecticide) was found in green dill (0.07 mg/kg) and green parsley (0.01 mg/kg) and was below MRL (1.0 mg/kg) as reported by FAO [29] . Farag et al. [30] reported that malathion was found in 18 samples in 17 different commodities from 4 countries, the highest value (1.21 mg/kg) recorded in eggplant.

Chlorpyrifos, as an organophosphate insecticide, was detected in three types of vegetables and the highest level was recorded in green parsley (1.74 mg/kg) and was above MRL as 0.5 mg/kg [27]. Concerning the previous studies, chlorpyrifos was detected in 38 samples in 33 different commodities from 7 countries, and the highest level was recorded in pepper as 3.1 mg/kg [31]. Chlorfenapyr (0.01 mg/kg) and propargite (0.06 mg/kg) were detected only in tomato samples and were within MRL as 0.01 mg/kg for each according to Codex [32]. The levels of chlorfenapyr and propargite in our samples are near to those recorded previously [33].

One type of pesticides was detected for each radish (permethrin, 0.01 mg/kg) and pepper (chlorpropham, 0.01 mg/kg). Level of permethrin and chlorpropham were lower than MRL (0.05 mg/kg) according to USDA [18]. Finally, Chlorothalonil, dimethomorph, and azoxystrobin were found only in Green parsley samples as 1.3, 0.06, and 0.15 mg/kg, respectively. The level of chlorothalonil was above MRL, while dimethomorph was lower than MRL as 0.6 for each [34]. Concerning the level of azoxystrobin, it was recorded lower than MRL as 2 mg/kg [35].

Sungur and Tunur [36] investigated pesticide residues in vegetable and fruit samples in Turkey and they reported that 60% of tested strawberry samples contained pesticide residues also, 70% and 80% of investigated green pepper and red pepper contaminated with pesticide residues respectively. Loughlin et al. [37] reported that out of 135 fruit and vegetable samples tested for pesticide residues; 65% were positive for one pesticide at least. From the total tested samples, 29% were at or below specified MRLs, while 36% were above that threshold; nevertheless, according to the index of food quality of residues, 39% of the samples were deemed inadequate for consumption. Chlorpyrifos was the most frequently detected pesticide and was usually accompanied by at least one other compound.

Regarding the previous Egyptian studies, Farag et al. [30] evaluated the levels of pesticides in some vegetables collected from the local markets at Cairo governorate. They reported that strawberries recorded the highest contamination with different pesticides such as ethion, propargite, permethrin, profenofos, chlorpyrifos, and their mean levels were 0.034, 0.023, 0.033, 0.024, and 0.050 mg/kg, respectively. Meanwhile, only two types of pesticides were detected in onion (sulfur, L-Cyhalothrin) and pepper (sulfur, methomyl). Also, only carbendazim was detected in green beans. In contrast, pesticides were not detected in tomatoes, cucumber, lettuce, okra, and peas. Dogheim et al. [38] monitored the contamination levels of pesticides in Egyptian fruits and vegetables collected from six governorates. They noticed that; about 76.1% of the total analyzed samples had no detectable residues, 23.9% contained detectable residues, and 2.59% contained residues that exceeded maximum residue limits.

Also, Badr et al.[3] evaluated the levels of pesticides in Egyptian fruits and vegetables collected from Giza governorate. They reported that chlorpyriphos (0.26 mg/kg) and propamocarb (0.039 mg/kg) recorded the levels of pesticides in apple and orange, respectively. Meanwhile, profenophos recorded the levels of pesticides in tomato and cucumber as 0.56 and 0.28 mg/kg, respectively. Ibrahim et al. [33] estimated the pesticide residues in some vegetables collected from Egyptian local markets located in eight governorates. They concluded that the recorded negative samples were 19.4% and 27.9% for pepper and cucumber, respectively. Meanwhile, the recorded positive samples were 80.6% and 72.1% for pepper and cucumber, respectively.

3.2. Pesticide residues in soil samples

The soil samples collected from four Egyptian governorates were analyzed for the presence of pesticide residues. **Table (2)** and **Figure (2)** showed the pesticide residues in soil samples collected from Kafr El-Sheikh governorate. Pesticide residues were detected in 60% of the tested areas of Kafr El-Sheikh with chlorpyrifos as a predominant pesticide (the LOQ values are less than 0.01 mg/kg) while P,P, DDE recorded the highest pesticide concentration with 0.05 mg/kg. **Table (3) and Figure (2)** represented the data obtained from pesticides analysis for soil samples collected from El-Beheira governorate. The pesticides contamination level in

El-Beheira Governorate showed the lowest contamination level among the tested areas with 30%. The pendimethalin residues recorded the highest concentration with 0.71 mg/kg. It is also observed that the three fields cultivated with strawberry in Com- Hamada area recorded a high level of pesticide residues compared with the type and concentration of pesticide residues.

TABLE 2. Qualitative and quantitative analysis of pesticides in agricultural soil (clay soil) collected from Kafr El-Sheikh governorate

Site	Sample Number	Cultivated crop	Detected pesticide	Concentration (mg/Kg)
El-Hamol	1	Beet	Chlorpyrifos	<LOQ
	2	Wheat	Chlorpyrifos	<LOQ
			Dicofol	<LOQ
			P,P,DDE	0.05
			Diazinon	0.02
	3	Beet	Chlorpyrifos	<LOQ
	4	Wheat	Chlorpyrifos	<LOQ
			Dicofol	<LOQ
			P,P,DDE	0.05
			Diazinon	0.02
	5	Beet	Chlorpyrifos	<LOQ
Sakha	6	Wheat	Not detected	
	7		Not detected	
	8		Not detected	
	9		Not detected	
Baltim	10	Bean	Chlorpyrifos	<LOQ
			Pendimethalin	0.02
			Dicofol	<LOQ
			P,P,DDE	0.05

LOQ: Limit of quantification.

Levels of pesticide residues in soil samples collected from Giza governorates were represented in **Table (4) and Figure (2).** The contamination percent with pesticide residues in Giza governorate was 70% with pendimethalin as the most dominant pesticide and with metalaxyl as the highest concentration 0.07 mg/kg. It is worth mentioning that, the fields cultivated with fresh vegetables such as dill, lettuce, onion, and spinach showed various types of pesticide residues with different concentrations which may be due to heavy and misuse of pesticides during the growing and harvest period. **Table (5) and Figure (2)** showed the data obtained from pesticides analysis of soil samples collected from Menoufia governorate. Pesticide residues were detected in 60% of tested areas in Menoufia governorate with difenoconazole as a predominant pesticide (the LOQ values are less than 0.01 mg/kg) while chlorfenapyr recorded the highest pesticide concentration with 0.02 mg/kg

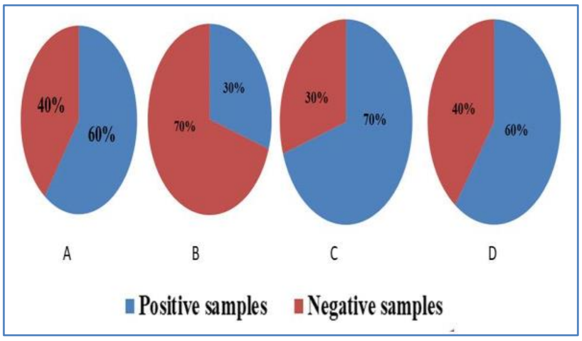

Fig. 2. Positive and negative percentage of pesticides residues incidence in soil samples collected from (A) Kafr El-Sheikh, (B) El-Beheira, (C) Giza, and (D) Menoufia governorates.

TABLE 3. Qualitative and quantitative analysis of pesticides in agricultural soil (clay soil) collected from El-Beheira Governorate

Site	Sample Number	Cultivated crop	Detected pesticide	Concentration (mg/Kg)
Al-Delengat	1	Peas	Not detected	
	2	Peas	Not detected	
Com-Hamada	3	Strawberry	Pendimenthalin	0.71
			P,P,DDE	<LOQ
			Cyhaltothrin Lambda	<LOQ
			Myclobutanil	0.02
			Boscalid	0.07
			Dimethomorph	0.02
			Thiophanate-methyl	0.01
			Carbendazim	0.02
			Pyraclostrobin	0.01
			Carbofuran	<LOQ
			Acetamiprid	<LOQ
	4	Strawberry	Boscalid	0.07
			Dimethomorph	0.02
			Thiophanate-methyl	0.01
			Carbendazim	0.02
			Pyraclostrobin	0.01
			Carbofuran	<LOQ
			Acetamiprid	<LOQ
	5	Strawberry	Dimethomorph	0.02
			Thiophanate-methyl	0.01
			Carbendazim	0.02
			Carbofuran	<LOQ
Damanhur	6	Peas	Not detected	
	7	Peas	Not detected	
	8	Peas	Not detected	
	9	Peas	Not detected	
	10	Peas	Not detected	

LOQ: Limit of quantification.

As illustrated in **Figure (2)**; the obtained data revealed that Giza governorate recorded the highest level of pesticide residues contamination with 70% positive samples followed by Kafr El-Sheikh and Menoufia governorates with 60 % positive samples and finally El-Beheira governorate recorded the lowest level of pesticide residues contamination with 30 % positive samples. The variation of pesticide levels between the current soil samples may be returned to many factors that play an important role in the persistence and degradation of pesticides in the soil such as soil characteristics and soil environmental factors [39]. Also, levels of pesticides in soil depend on their levels in irrigation water as reported by many Egyptian studies who noticed that levels of pesticides in River Nile water varied according to the type of irrigation water and level of water pollution [40-43].

Levels of pesticides in soil depend on the sampling site, in this respect, El-Kabbany et al. [40] reported that levels of chlorpyrifos (mg/kg) in soil samples collected from El-Zomor, El-Maryotia, and Kafer-Hakim at Giza governorate were 0.017, 0.019, and below the detection limit, respectively as well as levels of diazinon soil samples were below the detection limit, 0.014 and 0.021 mg/kg at the same order. Moreover, misuses of agricultural pesticides in the different sites lead to an increase in pesticide levels in the agricultural soil [44]. Also, levels of pesticides in soil samples varied according to season and depth of sampling [45].

TABLE 4. Qualitative and quantitative analysis of pesticides in agricultural soil (clay soil) collected from Giza Governorate

Site	Sample Number	Cultivated crop	Detected pesticide	Conc. (mg/Kg)
Atfih	1	Clover	Not detected	
Al-Shoubak	2	Clover	Chlorpyrifos	0.01
Al-Baragil	3	Drew	Pendimethalin	<LOQ
			P,P,DDE	<LOQ
Abu-Dahshan	4	Wheat	Not detected	
	5	Bean	Not detected	
Kafr Hakim	6	Lettuce	Difenconazole	0.03
			Pendimethalin	<LOQ
	7	Spinach	Pendimethalin	0.01
			Metalaxy	0.07
Nahia	8	Dill	Penconazole	0.01
			Cypermenthrin	0.01
			Pendimethalin	0.02
			Propargite	0.01
			Linuron	0.01
			Butralin	<LOQ
	9	Onions	Penconazole	<LOQ
			Cypermenthrin	0.01
			Pendimethalin	0.01
			Chloropyifos	<LOQ
	10	Clover	Malathion	<LOQ

LOQ: Limit of quantification.

TABLE 5. Qualitative and quantitative analysis of pesticides in agricultural soil (Sandy soil) collected from Khatatba site at Menoufia Governorate

Sample Number	Cultivated crop	Detected pesticide	Concentration (mg/Kg)
1	Onions	Difenoconazole	<LOQ
		Chlorfenapyr	0.01
2	Wheat	Difenoconazole	<LOQ
		Chlorfenapyr	0.01
3	Onions	Difenoconazole	<LOQ
4	Wheat	Difenoconazole	<LOQ
		Chlorfenapyr	0.02
5	Wheat	Not detected	
6	Wheat	Not detected	
7	Wheat	Difenoconazole	<LOQ
8	Onions	Not detected	
9	Onions	Not detected	
10	Wheat	Chlorfenapyr	0.01

LOQ: Limit of quantification.

Conclusion

Pesticide contamination is considered a serious problem that affects human health as well as causes economic losses and a bad reputation for agricultural products. The monitoring and awareness programs are required to illustrate the risk of misuse of pesticides. The present study concluded that many types of pesticide residues were detected in soil samples as well as fresh-cut vegetables with different levels according to the type of vegetable and source of soil sample. Soil samples of Giza governorate recorded the highest level of pesticide residues contamination with 70% positive samples. About 46.7 % of Giza vegetable samples showed pesticide residues with different types and concentrations. Propiconazole in green dill recorded the highest level as 3.8 mg/kg.

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Conflicts of Interest

The authors declare no conflict of interest.

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