



Article

The role of organic amendments in suppressing *Fusarium solani*, the causal agent of root rots in watermelon plants

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Abstract

This study aimed to evaluate the effect of organic amendments (vermicompost, fermented cow manure, and peat moss) in reducing root rot disease in watermelon caused by the fungus *Fusarium solani*. The most pathogenic isolates were identified morphologically and molecularly. Results showed significant differences in plant protection when organic amendments were mixed with soil at ratios of 1:1 and 1:2 along with the pathogenic fungus. Disease incidence and severity indices for vermicompost treatment at a 1:1 ratio were 42.85%, 0.61, and 30.95, respectively, compared to 100%, 3.80, and 94.04 for the pathogen-only treatment. The amendments also enhanced root growth, length, and fresh and dry weights compared to the pathogen treatment alone. Additionally, a significant increase in Peroxidase enzyme activity was observed in watermelon plants treated with organic amendments over three-time intervals (7, 14, and 21 days). The study highlights the role of organic amendments in promoting plant growth and mitigating disease.

Keywords: watermelon diseases; soil fungi; resistance; Sustainable agriculture.

دور المدعمات العضوية في تثبيط الفطر *Fusarium solani* المسبب لمرض تعفن جذور نباتات الرقي

نيران حسين علي و درين صفوت جميل

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المستخلص

هدفت الدراسة إلى تقييم تأثير المدعمات العضوية (الفيرموكومبوست، دمن الأبقار المخمر، والبتموس) في الحد من مرض تعفن جذور نبات الرقي الناتج عن الفطر *Fusarium solani* تم عزل وتشخيص العزلات الأكثر أمراضية مظهرياً وجزئياً. أظهرت النتائج فروقاً معنوية في حماية النبات عند استخدام المدعمات العضوية بنسب خلط (1:1) و(1:2) مع التربة والفطر الممرض. بلغت نسبة الإصابة ومعايير شدة المرض في معاملة الفيرموكومبوست عند خلط 1:1 (42.85% و0.61 و30.95) مقارنة مع معاملة الفطر الممرض (100% و3.80 و94.04). كما حسنت المعاملات نمو الجذر وطوله ووزنه الرطب والجاف مقارنة بمعاملة الفطر فقط. بالإضافة إلى ذلك، لوحظت زيادة معنوية في نشاط إنزيم Peroxidase في نباتات الرقي المعاملة بالمدعمات العضوية على ثلاث مراحل زمنية (7، 14، و21 يوماً). تُبرز الدراسة دور المدعمات العضوية في تعزيز نمو النبات والحد من المرض.

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1. Introduction

The watermelon plant (*Citrullus lanatus*) belongs to the family Cucurbitaceae, which comprises 120 genera and 750 species (1). It is considered one of the ten most economically significant vegetable crops worldwide (2). Iraq ranks sixth among Arab countries in watermelon production. According to statistical data from the Food and Agriculture Organization (3), global watermelon production reached 99.95 million tons, covering an area of 2,916,449 hectares. In Iraq, production amounted to 440,149 tons over an area of 23,733 hectares. Watermelon is susceptible to various diseases, including seed rot, seedling damping-off, leaf spot, stem gummosis, vascular wilt, root rot, and fruit rot (4, 5). The fungus *Fusarium solani* is among the pathogenic fungi characterized by its ability to produce a large number of inoculum units in the soil. It can remain viable in the absence of a host, making the management of soil-borne pathogens one of the most significant challenges for modern agricultural crop production systems (6, 7). Watermelon plants are particularly affected by several soil fungi that attack their roots, such as *F. solani*, during various growth stages (8, 9, 10).

The presence of organic matter in the soil enhances the activity of beneficial soil microorganisms, which contribute to protecting plants from diseases and mitigating their harmful effects (11). Peat moss has been used to induce resistance against *Fusarium* crown and root rot in tomatoes caused by *Fusarium oxysporum* f. sp. *lycopersici* and *Pythium oligandrum*. Results demonstrated reduced disease incidence and increased plant resistance to the pathogen (12). Additionally, mixing peat moss with soil reduced the development rate of *Fusarium* wilt in melon (*Cucumis melo* L.), with plant resistance levels ranging from 25% to 75% in peat-treated soils (1). The use of fermented cow manure has been identified as a sustainable method for utilizing natural resources to protect and improve the physical and chemical properties of soil, increase biodiversity in the environment, strengthen plant tissues and cells, and enhance plant resistance (13, 14). Cow manure is employed in biological control, organic fertilization, and growth promotion. It facilitates sulfur oxidation, phosphorus solubilization, and zinc uptake, playing a significant role in the biological processes of plants (15).

Vermicompost contains a wide variety of beneficial microorganisms that suppress several pathogenic agents. This has led to an increased focus on using vermicompost, which is rich in nutrients and enhances the physical, chemical, and biological properties of the soil, ultimately boosting agricultural productivity (16). Additionally, it promotes root growth and increases levels of gibberellic acid (GA3), abscisic acid (ABA), and indole acetic acid (IAA), which collectively enhance plant resistance to biotic and abiotic stress (16). Studies on the use of vermicompost in beet plants have shown increased epidermis and cortex thickness as well as cambium ring development, making plants more resistant to pathogens (17).

This study aimed to evaluate the role of organic amendments, including vermicompost, fermented cow manure, and peat moss, in reducing root rot disease incidence in watermelon plants.

2. Materials and Methods

2.1. Isolation and pathogenicity testing of *Fusarium spp.* isolates on watermelon seeds (Sugar Baby Cultivar)

Samples of watermelon plants were collected from various regions in Iraq (Baghdad - Abu Ghraib, Anbar - Amiriya Fallujah, Diyala - Khalis, and Salah al-Din - Ishaqi and Dujail) during the 2022 growing season. These samples exhibited symptoms of yellowing and root rot. The pathogen was isolated from the samples, and the isolates were labeled as K48, A8, B1, B6, B17, B23, B24, B26, and B29. The pathogenicity of the isolates was tested using 300 g pots containing sterilized silty clay soil, which were inoculated with 5 mL of fungal suspension per pot at a concentration of 1×10^6 spores/mL. Four watermelon seeds were sown per pot, with five replicates for each isolate. Additional replicates were maintained as controls without fungal inoculation. Disease incidence was assessed 40 days after planting (18).

2.2. Molecular Identification of the Pathogenic Fungus Isolate

The most pathogenic isolates of the fungus, obtained from watermelon plants showing symptoms of root rot disease, were identified molecularly. The isolates were cultured on water agar (WA) using the single-spore method. After incubation, the spores were transferred to Petri dishes containing potato dextrose agar (PDA) and incubated at $25 \pm 2^\circ\text{C}$ for 5 days.

Genomic DNA was extracted following the manufacturer's protocol provided with the extraction kit (Geneaid, South Korea). Polymerase chain reaction (PCR) was performed using the universal primers ITS1/ITS4. The samples were then submitted for nucleotide sequencing of the fungal isolates. The nucleotide sequences were deposited in the global GenBank database (<https://submit.ncbi.nlm.nih.gov/subs>) (19, 5).

2.3. Field testing of organic amendments for protecting watermelon plants from pathogenic fungi

A field experiment was conducted at a field of the College of Agricultural Engineering Sciences, University of Baghdad, following a Randomized Completely Block Design (RCBD). The experimental field was divided into 11 m $\times$ 3 m beds with 80 cm spacing between plants. The soil was inoculated with the most pathogenic fungal isolate by adding a spore suspension at a concentration of 1×10^6 spores/mL at a rate of 10 mL per planting hole. Three days after inoculation, organic amendments (vermicompost, fermented cow manure, and peat moss) were applied at mixing ratios of 1:1 (amendment:soil) and 1:2 (amendment:soil). Treatments also included the fungal pathogen, a fungicide, and a control, with three replicates for each treatment. Watermelon seedlings (Charleston Gray cultivar) at the three-leaf stage were transplanted during the spring season of 2023. Growth parameters were measured 120 days after planting, and the percentage of infection, disease index, and severity of infection were recorded at the end of the experiment (20).

2.4. Estimation of Peroxidase Enzyme Activity in Watermelon Plants for the Field Experiment

Peroxidase enzyme activity in watermelon treatments was measured following the method of (20). Measurements were taken at 7, 14, and 21 days after applying the treatments. Absorbance was recorded at 420 nm using a spectrophotometer every 30 seconds, with ten readings per sample. The rate of change in absorbance was used to determine enzyme activity.

3. Results

3.1. Isolation and pathogenicity testing of *Fusarium spp.* isolates on watermelon seeds (Sugar Baby Cultivar)

The results of isolation and identification of the fungus causing watermelon wilt disease indicated that *Fusarium solani* had the highest frequency of occurrence at 56%. This was followed by *Rhizoctonia solani* (17%), *Macrophomina phaseolina* (10%), *Aspergillus niger* (11%), *Ulocladium spp.* (3%), and *Trichoderma spp.* (1%) (Table 1).

The colonies of *Fusarium solani* were characterized by white or off-white mycelium, sometimes with a yellowish or pinkish tint. The hyphae were septate and simple. It was observed that *Fusarium solani* produces three types of conidia on branched conidiophores: macroconidia, which are multicellular; microconidia, which are spherical, oval, or elongated, transparent, and single- or double-celled, often forming small aggregates known as false heads; and chlamydospores, which are spherical or elongated, surrounded by a thick, smooth, or rough wall, and resistant to unfavorable environmental conditions for fungal growth and reproduction. Chlamydospores can be borne singly, in pairs, or in chains (Figure 1).

These characteristics align with the description of *Fusarium solani* provided by (21, 22, 23, 24). *F. solani* is considered one of the most harmful fungi affecting watermelon plants, causing an infection rate of no less than 73% of the total fungal infections reported in watermelon crops in Mexico City (25). It is among the most prevalent fungi in watermelon cultivation areas, causing root rot, basal stem rot, and plant wilt in Iraq (21, 26).

Table 1. The percentage frequency of fungi isolated from the roots and basal stems of infected watermelon plants.

Fungi	Frequency %
<i>Fusarium solani</i>	56%
<i>Rhizoctonia solani</i>	17%
<i>Aspergillus niger</i>	13%
<i>Macrophomina phaseolina</i>	10%
<i>Ulocladium spp.</i>	3%
<i>Trichoderma spp.</i>	1%

Each number in the table represents the average of three replicates

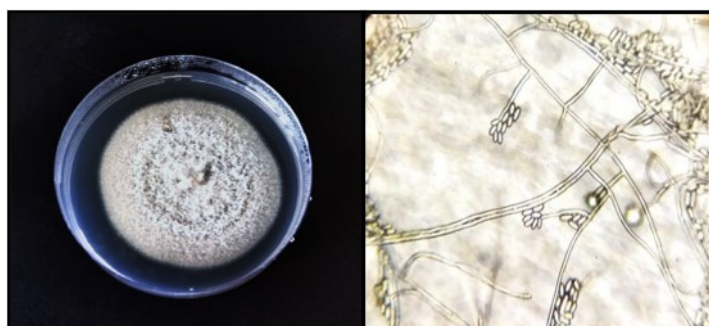


Figure 1. (A) Long conidiophore bearing microconidia, (B) Upper surface of the pathogenic fungal colony.

The pathogenicity test results showed significant variation among the *Fusarium solani* isolates in infection rates in watermelon seedlings (Table 2). The infection rates ranged from 25.00% to 83.33%. Isolate B6 exhibited the highest infection rate at 83.33%, followed by isolate B23 at 80.00%, and isolates B24 and B1, both at 75.00%. In contrast, isolate A8

had the lowest infection rate at 25.00% after 40 days of planting in pots, compared to the control treatment, which recorded 0.00% infection.

The high pathogenicity of certain isolates may be attributed to the genetic evolution of the fungus, enhancing its ability to produce various enzymes such as cutinase and pectinase. These enzymes degrade the plant cell wall, facilitating fungal colonization in the soil as a saprophyte and enabling root rot and seedling death upon host presence (27). Another possible mechanism involves fungal hyphae penetrating through the plant cortex into the xylem and phloem vessels, leading to vessel blockage. This induces tyloses formation as a plant defense response or fungal secretion of Fusaric acid, which damages plant cell membranes, increases reactive oxygen species in mitochondria, disrupts ATP production, and ultimately causes wilting and death of watermelon seedlings (26).

Table 2. Percentage of watermelon seed infection by *Fusarium solani* isolates in the pot experiment.

Fungus isolates <i>Fusarium solani</i>	Frequency %
A8	25.00
B1	75.00
B6	83.33
B17	58.33
B23	80.00
B24	75.00
B26	50.00
B29	58.33
K48	66.67
Control	0.00
LSD	12.78

Each number in the table represents the average of five replicates, with each replicate containing four watermelon seeds of the *Sugar Baby* cultivar.

3.2. Molecular Identification of the Pathogenic Fungal Isolate

The molecular analysis of the genomic DNA from two isolates of the pathogenic fungus *Fusarium solani* (B6 and B23) using the universal primers ITS4/ITS1 revealed the formation of bands with a molecular weight of 550 base pairs during electrophoresis (Figure 2).

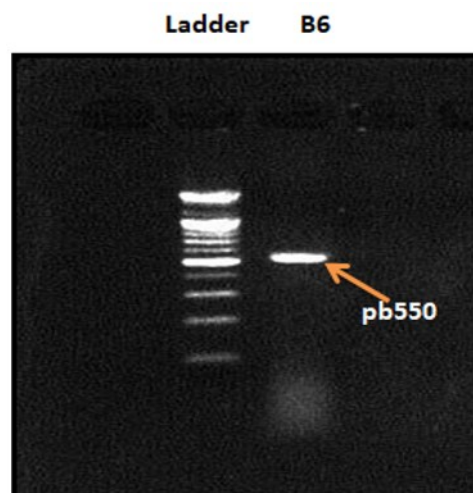


Figure 2. Electrophoretic migrations of nucleic acid for isolate B6 of the fungus *Fusarium solani*.

The nucleotide sequences of the ITS region of the pathogenic fungus were determined and compared with fungal isolates retrieved from the global GenBank database (<https://submit.ncbi.nlm.nih.gov/subs>). The comparison showed a 99.63–100% similarity with global *F. solani* isolates. The sequences of the fungal isolates were deposited in GenBank under accession numbers PP065521 and PP065522, respectively.

3.3. Effect of organic amendments on protecting watermelon plants from pathogenic fungi in the field.

The results of organic amendment treatments using vermicompost, fermented cow manure, and peat moss showed significant differences in protecting watermelon plants from the pathogenic fungus *Fusarium solani* (Table 3). For the 1:1 mixing ratio (organic amendment: soil) + pathogen, the disease incidence, disease index, and infection severity for the vermicompost treatment were 42.85%, 0.61, and 30.95, respectively. The values for the fermented cow manure treatment were 71.14%, 2.09, and 52.38, while for the peat moss treatment, they were 76.19%, 2.23, and 55.95, respectively. These were significantly lower than the control treatment with the pathogen, which recorded 100%, 3.80, and 94.04, respectively. For the 1:2 mixing ratio (organic amendment: soil) + pathogen, the vermicompost treatment recorded values of 57.14%, 1.76, and 44.04, respectively. The fermented cow manure treatment showed 85.71%, 2.47, and 61.90, while the peat moss treatment recorded 90.50%, 2.76, and 69.04, respectively. All treatments showed significant differences compared to the pathogen-only control (*F. solani*).

Table 3. Criteria for protecting watermelon plants from infection by the pathogenic fungus *Fusarium solani* in the field.

Treatment	Infection Percentage (%)	Disease Index	Infection Severity
1 Vermicompost : 1 Soil + <i>F. solani</i>	42.85	0.61	30.95
1 Vermicompost : 2 Soil + <i>F. solani</i>	57.14	1.76	44.04
1 Vermicompost : 1 Soil	38.09	0.57	14.82
1 Vermicompost : 2 Soil	52.37	1.14	28.57
1 Fermented Cow Manure : 1 Soil + <i>F. solani</i>	71.14	2.09	52.38
1 Fermented Cow Manure : 2 Soil + <i>F. solani</i>	85.71	2.47	61.90
1 Fermented Cow Manure : 1 Soil	57.14	1.47	38.27
1 Fermented Cow Manure : 2 Soil	71.42	2.04	51.19
1 Peat Moss : 1 Soil + <i>F. solani</i>	76.19	2.23	55.95
1 Peat Moss : 2 Soil + <i>F. solani</i>	90.5	2.76	69.04
1 Peat Moss : 1 Soil	71.43	1.9	47.61
1 Peat Moss : 2 Soil	85.71	2.19	54.76
Fungicide	85.71	2.66	66.6
Control (No Pathogen)	71.42	1.71	42.85
<i>F. solani</i> (Pathogen Treatment)	100	3.80	94.04
LSD 0.05	3.04	0.01	2.82

* Each number represents the average of three replicates, with each replicate containing four watermelon plants.

The organic amendments provided a positive effect on the proportion of healthy watermelon seedlings, protecting them from infection by the pathogenic fungus *F. solani* 30 days after planting in soil with organic amendments (vermicompost, fermented cow manure, and peat moss) in pathogen-contaminated fields. The average number of healthy plants at a 1:1 mixing ratio (organic amendment: soil) + *F. solani* was 7.00, 6.66, and 6.00

plants per treatment for vermicompost, fermented cow manure, and peat moss, respectively. These values showed significant differences compared to the *F. solani*-only treatment, which had 3.33 healthy plants.

At a 2:1 mixing ratio (organic amendment: soil) + *F. solani*, the average number of healthy plants was 6.66, 6.33, and 5.33 plants per treatment for vermicompost, fermented cow manure, and peat moss, respectively. These results differed significantly from the *F. solani*-only treatment, which had 3.33 healthy plants. (17) . (Table 4).

Table 4. Germination percentage and selected growth parameters of watermelon plants in the field.

Treatment	*Healthy Plants (%) After 30 Days	**Root Length (cm)	**Fresh Root Weight (g)	**Dry Root Weight (g)	**Shoot Length (cm)	**Branches Count	**Sexual Expression	**Fruit Count
1 Vermicompost : 1 Soil + <i>F. solani</i>	7.00	99	48.01	21.51	348	9.00	19	2.33
1 Vermicompost : 2 Soil + <i>F. solani</i>	6.33	99	42.12	14.14	355	7.77	16.6	2.00
1 Vermicompost : 1 Soil	7.00	99	65.47	25.61	279	9.5	39	2.5
1 Vermicompost : 2 Soil	7.00	88	45.03	16.83	244	8.17	29.88	2.2
1 Fermented Cow Manure : 1 Soil + <i>F. solani</i>	6.67	80	37.26	9.27	300	7.17	15.05	1.9
1 Fermented Cow Manure : 2 Soil + <i>F. solani solani</i>	6.33	62	40.16	7.87	240	6.93	13.25	1.33
1 Fermented Cow Manure : 1 Soil	6.33	71	26.4	9.44	283	6.67	20.63	1.33
1 Fermented Cow Manure : 2 Soil	6.33	86	23.67	9.28	316	6.00	18.25	1.67
1 Peat Moss : 1 Soil + <i>F. solani</i>	6.00	58	24.59	6	242	6.33	14.63	1.00
1 Peat Moss : 2 Soil + <i>F. solani</i>	5.33	40	24.12	4.75	200	6.00	13.03	1.00
1 Peat Moss : 1 Soil	6.33	75	38.26	8.27	293	5.67	17.36	1.33
1 Peat Moss : 2 Soil	6.00	78	35.73	8.86	303	5.93	16.85	1.33
Fungicide	3.33	32	23.19	9.3	275	6.33	13.63	1.67
Control (No Pathogen)	6.00	19	26.91	9.79	204	6.27	13.75	1.00
<i>F. solani</i> (Pathogen Treatment)	5.33	29	12.64	4.05	158	5.33	5.00	0.67
LSD 0.05	0.85	1.2	1.21	0.85	12.3	1.12	0.12	0.63

*Each number represents the average of three replicates, with each replicate containing seven watermelon plants.

**Each number represents the average of three replicates, with each replicate representing the average of four watermelon plants.

3.4. Effect of Organic Amendments on Peroxidase Activity in Watermelon Plants in the Field Experiment

The addition of organic amendments to the soil significantly increased the induction of peroxidase enzyme activity in watermelon plants compared to the treatment with the pathogenic fungus. Peroxidase activity was measured based on the rate of change in optical absorbance per minute per gram of fresh weight, and the enzyme's activity was evaluated at three intervals: 7 days, 14 days, and 21 days after the treatments were applied (Table 5). In the first week, the enzyme activity for the 1:1 mixing ratio (organic amendment: soil) + pathogen was significantly higher in treatments with vermicompost, fermented cow manure, and peat moss, reaching 66.8, 53.1, and 55.0 $\Delta\text{abs}/\text{min}/\text{g}$ fresh weight, respectively. These values were significantly higher than the enzyme activity in the pathogen-only treatment, which recorded 44.2 $\Delta\text{abs}/\text{min}/\text{g}$ fresh weight.

After 14 days of applying the treatments, the highest peroxidase activity levels were recorded in the vermicompost, fermented cow manure, and peat moss treatments, with values of 75.2, 64.3, and 64.3 $\Delta\text{abs}/\text{min}/\text{g}$ fresh weight, respectively, compared to 45.8 $\Delta\text{abs}/\text{min}/\text{g}$ fresh weight in the pathogen-only treatment. By the 21st day, peroxidase activity in the vermicompost, fermented cow manure, and peat moss treatments decreased to 60.4, 48.5, and 50.1 $\Delta\text{abs}/\text{min}/\text{g}$ fresh weight, respectively, but these values remained significantly higher than the enzyme activity in the pathogen-only treatment, which recorded 39.5 $\Delta\text{abs}/\text{min}/\text{g}$ fresh weight. The increase in peroxidase enzyme activity in watermelon plants treated with organic amendments can be attributed to the stimulatory effects of these amendments on the plant's defense mechanisms. Peroxidase is one of the most effective enzymes in plant tissues, contributing to suberin deposition and strengthening cell walls against pathogens and abiotic stress factors. It also plays a pivotal role in activating plant defense responses against pathogens by facilitating ethylene production and inducing phytoalexin synthesis (2, 28). The findings of this study align with those of (29), who reported that the use of the organic amendment vermicompost increased peroxidase enzyme activity at mixing ratios of 5% and 10%, providing resistance to plants against the pathogenic fungus *Rhizoctonia solani*, the causal agent of root rot in beans.

Table 5. Effectiveness of the organic amendment vermicompost on peroxidase enzyme activity ($\Delta\text{abs}/\text{min}/\text{g}$ fresh weight) in watermelon plants infected by *Fusarium solani*, the causal agent of wilt and root rot in watermelon.

Treatment	After 7 days	After 14 days	After 21 days	Average
1 Vermicompost : 1 Soil + <i>F. solani</i>	66.8	75.2	60.4	67.47
1 Vermicompost : 1 Soil	70.6	73.3	68.8	70.9
1 Fermented Cow Manure : 1 Soil + <i>F. solani</i>	53.1	64.3	48.5	55.3
1 Fermented Cow Manure : 1 Soil	50.1	57.5	50.5	52.7
1 Peat Moss : 1 Soil + <i>F. solani</i>	55	64.3	50.1	56.47
1 Peat Moss : 1 Soil	48.2	55.3	50.0	51.17
Fungicide	43.0	44.7	40	42.57
Control (No Pathogen)	38.1	39.2	38.0	38.43
<i>F. solani</i> (Pathogen Treatment)	44.2	45.8	39.5	43.17
Average	52.12	57.73	49.53	53.13
LSD 0.05	0.802	0.802	0.802	0.519

* Each number represents the average of three replicates, with each replicate consisting of a sample from one plant.

4. Discussion

The current study highlights the significant role of organic amendments, including vermicompost, fermented cow manure, and peat moss, in enhancing watermelon plant resistance against *Fusarium solani*, the causal agent of root rot and wilt disease. The amendments improved plant health by increasing the activity of peroxidase enzyme, a critical component of the plant's defense system, and strengthening root and shoot growth. Vermicompost demonstrated the highest efficacy in reducing disease severity and promoting plant vigor, followed by fermented cow manure and peat moss. These findings underline the potential of integrating organic amendments into sustainable agricultural practices to mitigate soil-borne diseases, reduce reliance on chemical pesticides, and improve crop productivity. This approach not only offers an eco-friendly alternative for disease management but also contributes to the long-term health of soil ecosystems. Future research

should explore the mechanisms underlying these effects and evaluate the scalability of these practices under diverse field conditions.

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Conflicts of Interest: The authors declare no conflicts of interest.

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