Research Article

Comparative Effects of Entomopathogenic Nematodes, Biopesticides, and Plant Extracts Against Agricultural Pest *Meloidogyne incognita* (Tylenchida: Heteroderidae)

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Abstract | This study investigates the availability and efficacy of biopesticides, specifically neem oil, and entomopathogenic nematodes (EPNs) in urban agriculture within Karachi, Sindh, Pakistan. A survey conducted across twenty local markets revealed that neem oil was the only biopesticide available, highlighting a significant gap in the accessibility of eco-friendly pest management options amidst prevalent chemical pesticide use. Soil samples collected from various plant types on the University of Karachi campus successfully isolated *Steinernema pakistanense* nematodes, indicating their potential as effective biological control agents. The inhibitory effects of neem oil, *Syzygium cumini* leaf extract, and EPNs on egg hatching were evaluated, revealing that neem oil exhibited the highest efficacy with 98% inhibition at 72 hours; however, no statistically significant differences were noted among treatments over time. Additionally, a nursery trial assessed the impact of these treatments on plant growth parameters. EPNs demonstrated superior performance in promoting plant height and root growth compared to other treatments, indicating their dual role in pest control and plant health. These findings underscore the potential of integrating biopesticides into pest management strategies to enhance sustainability in urban agriculture. Future research should focus on optimizing formulations and exploring synergistic combinations of biopesticides to maximize pest control efficacy and improve crop yield.

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Introduction

The growing dissatisfaction with synthetic chemical pesticides for managing agricultural pests stems from the global need to produce more food while ensuring its safety. Overuse of these chemicals has led to concerns about environmental pollution, health hazards, and pest resistance, prompting a shift toward safer, eco-friendly alternatives (Abd-El-Gawad *et al*., 2017; Ismail *et al*., 2020). This has driven researchers to explore biological control agents and biopesticides as more sustainable solutions. Biopesticides are a superior component of Integrated Pest Management (IPM) due to their specificity, minimal toxicity, low production costs, and environmental safety (Hajek, 2004). Although biopesticides are not typically used as standalone treatments, their integration with other methods in IPM programs enhances their effectiveness in pest management (Lacey and Shapiro-Ilan, 2008; Askary, 2015; Wang and Knodel, 2022).

The excessive use of synthetic pesticides, while initially improving crop protection (Oerke, 2006), now costs billions annually and causes ecological imbalances (James, 2007). Pakistan alone spent US$183 million on pesticides in 2006 (Tariq *et al*., 2007), and the negative effects of these chemicals are well-documented, including pest resistance, environmental pollution, and health risks (Pimentel and Peshin, 2014). Consequently, many countries are promoting biopesticides as part of their pest management strategies, as biocontrol agents are eco-friendly alternatives that are often pest-specific and effective when pests become resistant to traditional pesticides (Shahina *et al*., 2017).

Entomopathogenic nematodes (EPNs), specifically from the Steinernematidae and Heterorhabditidae families, have emerged as highly effective biocontrol agents. These nematodes, in mutualistic association with Xenorhabdus and Photorhabdus bacteria, are lethal to soil-dwelling arthropods (Stock, 2005). EPNs enter their hosts through natural openings, release symbiotic bacteria that kill the pest within 48 hours, and are highly pathogenic to a wide range of insect pests (Shapiro-Ilan and Gaugler, 2015). However, their efficacy can vary between laboratory and field conditions, likely due to environmental factors (Grewal *et al*., 2005). EPNs are ideal for IPM programs as they are non-toxic to humans, highly specific to target pests, and compatible with conventional pesticide application methods (Shapiro-Ilan *et al*., 2006; Lewis and Kaya, 2021).

Biopesticides, including plant-based products, are increasingly recognized for their potential to provide sustainable crop protection. The public's growing awareness of pollinator decline and environmental conservation has fueled interest in biopesticides (Miller, 2004). Many small companies now produce natural pest control products using essential oils and other bioactive compounds from plants. These products are often of food-grade quality and do not require extensive toxicological testing, making them commercially viable for large-scale agricultural use (Glare *et al*., 2012; Jabeen *et al*., 2023). Moreover, biopesticides are economically favorable, particularly for small-scale farmers with limited financial resources (Huerga and San Juan, 2005).

Plant extracts are also gaining attention for their insecticidal properties. Plants contain bioactive compounds that serve as natural defenses against pests, making them a promising alternative to synthetic pesticides (Caboni *et al*., 2012). These botanical insecticides are specific to target species, environmentally safe, and suitable for use in IPM programs (Pavela, 2016). However, the commercial development of botanical pesticides remains limited due to regulatory challenges and competition from other products (Walia and Koul, 2008; Zhang *et al*., 2022).

Root-knot nematodes (*Meloidogyne* spp.) are among the most destructive pests affecting crops globally. These nematodes have wide host ranges, high reproductive rates, and can cause severe economic losses (Perry *et al*., 2009). Effective management of root-knot nematodes requires a combination of control practices, including cultural methods, host plant resistance, and biological control agents (Nyczepir and Thomas, 2009). As traditional chemical nematicides are being phased out due to environmental and health concerns, alternative strategies, such as biocontrol agents, are becoming increasingly important for managing these pests (Batchelor, 2002; Mwangi *et al*., 2021).

Materials and Methods

Local market surveys for biopesticide

A survey of local markets in Karachi, Sindh, Pakistan, was conducted to assess the availability of biopesticides. Twenty different markets were surveyed. The purpose of this survey was to gather information on the types of biopesticides available and to ensure that these markets stocked necessary products for biological pest control research.

Isolation and collection of entomopathogenic nematodes (EPNS) as biocontrol agents

A total of 20 soil samples, with two samples collected from each site, were obtained from various locations within the University of Karachi, Karachi, Pakistan. The collection sites included the National

Nematological Research Center (NNRC) field (lawn grass), NNRC nursery (ornamental plants), botanical garden (lawn grass), university campus road (rose plants), Crop Disease Research Institute (CDRI, agriculture field), Department of Agriculture and Agribusiness (agriculture field), Pakistan Agriculture Research Council (PARC, agriculture field), and the Departments of Botany (lawn grass) and Zoology (rose plants). The purpose of the sampling was to isolate entomopathogenic nematodes (EPNs) from different habitats. Each soil sample was placed in a separate plastic bag, secured with an elastic band, and labeled with the relevant details, including the locality, host, and date of collection. The samples were transported to the laboratory under controlled conditions (10°C) for nematode extraction. Entomopathogenic nematodes (EPNs) were extracted from soil samples using Galleria mellonella (Greater Wax Moth, Linnaeus) larvae, which serve as an ideal host due to their susceptibility to nematode infection, ease of rearing, and availability. The wax moth larvae were sourced from the rearing laboratory at the National Nematological Research Centre (NNRC), University of Karachi, Sindh, Pakistan.

Formulation of extracts

Fresh, healthy leaves of the jamun plant (*Syzygium cumini* (L.)-Myrtaceae) were collected from the Department of Agriculture and Agribusiness at the University of Karachi. The leaves were thoroughly washed under running tap water and placed in a shaded area to air dry for 6-7 days. Once dried, the leaves were ground into a fine powder using an electric grinder. A quantity of 20 grams of the powdered leaves was soaked individually in 100 ml of distilled water for 24 hours in 1000 ml Erlenmeyer flasks. After this soaking period, the mixture was filtered through cheesecloth to remove solid residues. The resulting filtrate was then centrifuged for 10 minutes at 4000 revolutions per minute (rpm) to prepare it for use in experimental trials. The concentrated extract was designated as "SS" for standard stock and was stored at a temperature of 15–18 °C for subsequent experimental applications. For the nursery pot trial, the filtrate obtained from the cheesecloth was used directly, and the centrifugation step was skipped.

In vitro experiment

Root-knot nematodes (RKN), *Meloidogyne incognita*, were isolated from infested *Solanum lycopersicum* (tomato) plants with characteristic galling symptoms. These pure cultures were maintained in the greenhouse of the National Nematological Research Centre (NNRC), University of Karachi. To further confirm the root-knot nematodes, perennial pattern analysis was conducted (Perry *et al*., 2009).

Infected tomato plants were uprooted and thoroughly washed with spring water to eliminate any adhering soil. Mature female nematodes were extracted from the root tissues using fine forceps and a needle. The collected females were immediately placed in a 45% lactic acid ($C_3H_6O_3$) solution. After a 24-hour period, the internal body contents were carefully removed using needles. The posterior half of each female was then cut and mounted on a slide in lactophenol for microscopic examination at 40x magnification. The cuticle surrounding the perennial pattern was meticulously trimmed to facilitate accurate positioning under the microscope, ensuring permanent mounting in a drop of lactophenol.

Extraction of egg masses

Tomato plants infected with RKN from the established pure culture were excavated and gently cleaned under flowing water. The roots were cut into segments approximately 2-3 cm in length and placed in a clean Petri dish for observation under a stereomicroscope to identify egg masses. Egg masses of *Meloidogyne incognita* were carefully retrieved from the infected roots using forceps and needles and then transferred into a clean glass cavity block.

Evaluation of treatments on egg hatching inhibition

To assess the effects of different treatments on egg hatching, three concentrations of jamun leaves extract and neem oil were prepared: 1%, 3%, and 5% (diluted with distilled water). In parallel, different concentrations of *S. pakistanense* juveniles (50, 100, and 150 juveniles/mL) were counted and collected in a sterilized beaker. The experimental design adopted was a completely randomized design (CRD) with five replicates. A single egg mass from the root-knot nematode was placed separately into 3 mL of each of the three concentrations of leaves extract, neem oil formulation, or *S. pakistanense* juveniles in sterilized Petri dishes measuring 5 mm in diameter. For the control treatment, egg masses were incubated solely in spring water without any applied treatments. All experimental Petri dishes were incubated at 25 °C. Egg hatching inhibition and the number of hatched juveniles were recorded after 72 hours of incubation.

The hatched juveniles were counted using a Nikon stereomicroscope at 4x magnification. Egg masses were retained in the Petri dishes, and treatments from each dish were transferred to a nematode counting plate (Thomas Scientific) to calculate the mean value of egg inhibition.

Nursery trial

Ten seeds of mung bean *Vigna radiata* (Fabaceae) were thoroughly washed, air-dried, and sown in sandy loam soil mixed with farmyard manure at a ratio of 3:1:1. The soil was sterilized at 50 °C to eliminate any contaminants. The pots were disinfected with a 0.4% formalin solution. Once cooled, 1 kg of the sterilized soil was placed into 15 cm diameter earthen pots. To prepare for the natural hatching of the nematodes, 50 egg masses of *M. incognita* were carefully collected using forceps and a needle droplet pipette. These were placed in Petri dishes filled with spring water at room temperature (28±2°C) for 48 hours to allow the eggs to hatch. The freshly hatched second-stage juvenile (J2) nematodes were then transferred to a 250 mL Erlenmeyer flask, where their concentration was measured using a counting dish under a dissecting microscope. A 3mL aliquot of the nematode suspension was mixed thoroughly and dispensed into the counting dish, with the total number of juveniles counted using systematic gridlines for accuracy. The average count was calculated over three repetitions multiplied by the total volume of the suspension.

Experimental design and treatment applications

The experiment was set up using a complete randomized block design (CRBD), with three replications and two concentrations for each treatment in the greenhouse at the National Nematological Research Centre, University of Karachi. After germination, the seedlings were thinned to two plants per pot and watered as needed. Each pot was inoculated with a suspension of 100 juveniles of M. incognita, which was carefully applied around the root zone after slightly lifting the seedlings out of the topsoil. Following inoculation, the soil was replaced and the plants resumed normal watering routines. One-week post-inoculation, treatments were applied in the form of 20 mL of two concentrations (3% and 5%) of neem oil and jamun leaves extract, along with biocontrol nematodes of *S. pakistanense* at concentrations of 200 and 300 IJs/mL. Two control treatments were established: Control 1, which received no nematode juveniles or treatments; and Control 2, which received 100 nematode juveniles without any treatments.

Data collection methods

After 40 days, the experiment was concluded, and various growth parameters were measured, including plant height, root length, and shoot length, all recorded in centimeters (cm). Fresh weights of both the roots and shoots were recorded in grams (g). The total number of galls on the infected roots was counted using a magnifying glass for comparative analysis. Additionally, soil samples from each pot were collected, and nematode populations were assessed using the sieving and decanting method followed by Baermann funnels.

Data analysis

The effects of the treatments were analyzed using a multifactor ANOVA, and significant differences in means were determined through Duncan's Multiple Range Test (DMRT) at a significance level of $P<0.05$, as outlined by SAS Institute (2002).

Results and Discussion

Surveys of local market

A survey was conducted across twenty different local markets in Karachi, Sindh, Pakistan, specifically targeting areas such as M. A. Jinnah Road, Aram Bagh, Saddar, Newtown, Lee Market, Nazimabad, the Old City area, Sohrab Goth, Kharadar Bunder Quarter, Soldier's Bazaar, Jinnahabad, Jamia Masjid Kemari, Saudabad, Timber Market (Old Haji Camp), Jodia Bazaar, and Sarafa Bazaar (Napper Quarter). Out of these twenty markets, only one chemical and biochemical market located at Old Sabzi Mandi offered a biopesticide called Neem Oil Hara. The majority of the markets primarily featured chemical pesticides.

The findings from the survey conducted in Karachi highlight a significant gap in the availability of biopesticides, with neem oil representing the only biopesticide option in chemical and biochemical markets across 20 different localities. Despite recognizing the effectiveness of biopesticides such as neem oil, their limited availability suggests a need for greater market integration and awareness surrounding their benefits for sustainable agriculture (Kumar *et al*., 2019). Neem oil is renowned for its potential in pest management due to its active compounds, such as azadirachtin, which have both insecticidal and growth-regulating properties (Srinivasan, 2018). Recent studies have also shown that increased awareness and promotion of biopesticides can lead to higher adoption rates among farmers (Kumar and Singh, 2022). The limitations in the availability of biopesticides highlight a broader trend observed globally, where ecological alternatives often lag behind conventional chemical options. The lack of market presence not only restricts farmers' choices but also perpetuates reliance on synthetic chemicals, which can have detrimental environmental impacts (Zhao *et al*., 2019). Although neem oil has demonstrated insecticidal and growth-regulating properties (Isman, 2006), its current limited availability in urban markets poses significant challenges for sustainable pest control practices.

Entomopathogenic nematodes

Soil samples were collected from various sources, including banana trees, lawn grass, ornamental plants, rose plants, brinjal, and agricultural fields. A total of twenty samples were taken from ten different locations across the main campus of the University of Karachi, Karachi, Pakistan. Among these samples, entomopathogenic nematodes identified as *Steinernema pakistanense* were confirmed based on taxonomic characterization of the first-generation male, female, second-generation male, female, and infective juveniles, following the de Man, 1884 formula confirmed with the original description.

In exploring the efficacy of EPNs, our identification of *Steinernema pakistanense* reinforces the diversity and potential of indigenous nematodes in biocontrol efforts (Huang *et al*., 2020). The substantial differences in plant height and shoot length observed in the nursery trial substantiate the potential of EPNs to enhance plant health compared to conventional treatments. Previous studies have reported similar results, where EPNs not only reduced nematode populations but also improved plant vigor (Yang *et al*., 2017; Elawad *et al*., 2015). Additionally, a recent review by Garcia *et al*. (2023) emphasizes the role of EPNs in integrated pest management, particularly in relation to their compatibility with organic farming practices. Notably, our results align with previous findings indicating that EPNs can produce superior control of *M. incognita* compared to botanical treatments (Sharma *et al*., 2020).

Egg hatching

Previous studies have explored similar topics concerning nematode egg hatching. For instance, research has shown that natural extracts, such as those from *Azadirachta indica* (Neem), possess nematicidal properties (Khan *et al*., 2010). The mechanisms through which these extracts inhibit hatching could involve disruption of the nematode's hormonal regulation or interference with egg structure. A recent study by Nazir *et al*. (2023) highlights the molecular mechanisms by which neem extracts induce nematode mortality, providing insights into their potential use as effective biopesticides. Additionally, studies emphasize the potential of plant-derived extracts as environmentally friendly alternatives to synthetic nematicides, aligning with current trends toward sustainable agriculture (Dawood *et al*., 2016).

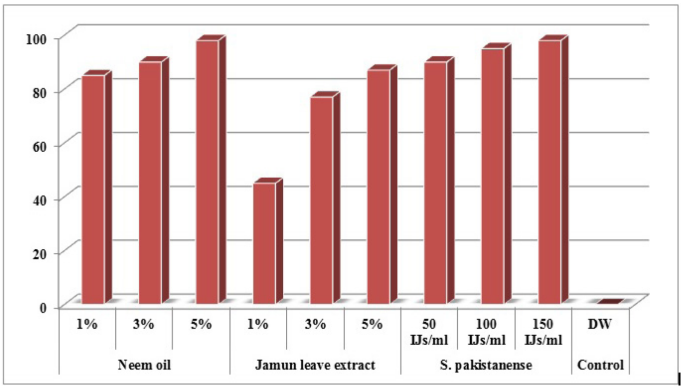

Figure 1: *Comparative effect of entomopathogenic nematodes, leaves extract and bio pesticide on egg hatching.*

The analysis of variance (ANOVA) was conducted to evaluate the differences in egg hatching inhibition among the treatments: Neem oil, jamun leaf extract, and *S. pakistanense* (Figure 1). The ANOVA results indicated no statistically significant differences among the treatments ($F(2, 6) = 2.9815$, $p = 0.1262$), with an effect size (η^2) of 0.4985, suggesting a moderate effect. These results indicate that while there are observable differences in mean inhibition rates, they are not statistically significant at the 0.05 level. The effect size suggests a moderate impact of treatment type on egg hatching inhibition, warranting further investigation with larger sample sizes. Recent findings by Ali *et al*. (2024) indicate that varying the concentration of biopesticides can enhance their efficacy against RKNs, suggesting dosage optimization could lead to more significant results.

In contrast, previous literature also highlights varying susceptibility and responses of nematodes to different treatments, affected by factors such as the concentration of the extract, the developmental stage of the nematodes, and environmental conditions (Yang *et al*., 2020). Previous research also highlighted the potential of EPNs in controlling nematode populations, with studies demonstrating their ability to affect not only insect pests but also plant-parasitic nematodes, including RKN. For instance, Grewal *et al*. (2005) reviewed the utility of EPNs as biological control agents, indicating that these nematodes can exploit plant-parasitic nematodes, thereby inhibiting their growth and reproduction. Recent research by Martinez *et al*. (2022) further supports this finding, showing that EPNs exhibit promising control over nematode populations, contributing to sustainable agriculture. The mechanisms by which EPNs exert control may include direct predation on juvenile stages of nematodes or the secretion of metabolites that affect nematode egg hatching (Kaya and Gaugler, 1993).

Floyd *et al*. (1999) specifically assessed the impact of EPNs on the egg-hatching of *Meloidogyne incognita* and found that EPNs could significantly reduce hatching rates. These findings illustrate the potential for integrating EPNs into nematode management strategies, particularly in conjunction with plant extracts like neem and jamun leaves, known for their nematicidal properties (Khan and Grewal, 2003). The holistic use of both biocontrol agents could create an environment less conducive to RKN reproduction.

Nursery trial

Effect of treatments on plant growth parameters: Analysis of variance (ANOVA) revealed significant differences in plant height (F= 30.039, P= 0.0097) and shoot length (F= 22.143, P= 0.0150) among treatments. The highest plant height was recorded in *S. pakistanense* treatments (48-51 cm), followed by neem oil (42-45 cm) and jamun leaves extract (38-40 cm), compared to the RKN-inoculated control (28 cm). Root length showed marginal differences among treatments (F= 6.619, P= 0.0775). Fresh weight parameters (root and shoot) did not differ significantly among treatments (P > 0.05). All treatments significantly reduced root galling (F= 19.723, P= 0.0177) and soil nematode population (F = 43.883, P= 0.0056) compared to the RKN-inoculated control. The control plants showed severe root galling (352 galls/plant) and a high nematode population (530 ± 15 *M. incognita*/100 g soil). *S. pakistanense* demonstrated the highest efficacy, with 300 IJs/mL reducing root galling by 86.36% (48 galls/plant) and soil nematode population by 93.40% (35±5 M. incognita/100 g soil). The 200 IJs/mL concentration showed similar but slightly lower efficacy (84.66% and 91.51% reduction in galling and soil population, respectively). Jamun leaves extract at 5% concentration reduced root galling by 82.39% (62 galls/plant) and soil nematode population by 84.34%. The 3% concentration showed marginally lower efficacy (79.83% and 81.89% reduction, respectively). Neem oil treatments showed moderate efficacy, with the 5% concentration resulting in a 76.70% reduction in root galling (82 galls/plant) and an 80.75% reduction in soil nematode population. The 3% concentration showed lower efficacy (53.12% and 64.15% reduction, respectively). The untreated control (distilled water) showed normal plant growth with no root galling or nematode infection, confirming the pathogenicity of *M. incognita* in the experimental conditions (Table 1). These results demonstrate that the entomopathogenic nematode *S. pakistanense*, particularly at 300 IJs/mL, provides superior management of *M. incognita* compared to botanical treatments while supporting better plant growth parameters.

Table 1: *Effect of treatments on growth parameters and Meloidogyne incognita infestation in plants.*

Treatment	Concentration	Plant height (cm)	Root length (cm)	Shoot length (cm)	Root fresh weight (g)	Shoot fresh weight (g)	No. of galls/ plant	No. of M. incognita/ 100g soil
Neem oil	3%	42	5	37	0.61	1.45	165	190
Neem oil	5%	45	6	39	0.4	2.2	82	102
Jamun leaves extract	3%	38	4	31	0.62	1.6	71	96
Jamun leaves extract	5%	40	5	35	0.04	2	62	83
S. pakistanense	200 IJs/ml	48	7	41	0.47	2.35	54	45
S. pakistanense	300 IJs/ml	51	8	43	0.4	2.4	48	35
Control 1	100 RKN larvae	28	5	23	0.78	2.1	352	530
Control 2	Distilled water	35	6	29	0.44	2.16	0	0

This finding corroborates established research demonstrating the potency of EPNs in managing nematode populations (Bigar *et al.*, 2017). Furthermore, our research reflects the importance of dosage, where higher nematode concentrations yield improved outcomes, emphasizing the significance of optimal application rates in biocontrol strategies (Grewal *et al.*, 2005). A recent meta-analysis by Khanna *et al.* (2023) further affirms this, indicating that the success rates of EPNs often correlate with the dosage applied in varied environmental conditions. Despite the moderate efficacy of neem oil and jamun leaves extract, their roles in integrated management systems cannot be understated. Neem oil offered considerable, albeit less potent, control of M. incognita, highlighting its potential as a tactical component of pest management programs in conjunction with EPNs (Abdel-Basset and Sabry, 2021).

The use of plant extracts and biopesticides is gaining traction as a means to enhance ecological resilience in agricultural ecosystems, promoting a reduction in chemical pesticide reliance (Hussain *et al.*, 2020). Additionally, the moderate effect size observed in the egg hatching inhibition analysis indicates room for enhanced formulations or combinations of treatments to achieve statistically significant differentiation. Investigations into synergistic applications involving multiple biopesticide approaches could yield promising results (Gomez *et al.*, 2019). Thus, further research is warranted to explore the interactions between biopesticides and their effects on pest populations, which could lead to improved strategies for sustainable agriculture.

Conclusions and Recommendations

This research highlights the significant potential of biopesticides, particularly neem oil and the indigenous entomopathogenic nematode *Steinernema pakistanense*, for managing root-knot nematodes in Karachi, Pakistan. Furthermore, the comparative analysis of neem oil and jamun leaf extract indicates that these natural alternatives can serve as effective adjuncts to EPNs, contributing to integrated pest management strategies. By promoting the adoption of such biopesticides, we can enhance not only crop health and yield but also support sustainable agricultural practices that minimize environmental impact. Future research should focus on optimizing the application rates and combinations of these biopesticides, as well as exploring additional natural products, to develop comprehensive management strategies that effectively combat nematode infestations. The integration of biopesticides into agricultural systems could be an important step toward fostering resilience in farming practices and ensuring food security in Pakistan.

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Novelty Statement

By highlighting that neem oil is the sole biopesticide accessible in local markets, this study emphasizes the critical necessity to diversify sustainable pest management options. Furthermore, this research promotes the incorporation of biopesticides into urban farming practices, paving the way for future investigations aimed at enhancing these solutions to boost both pest control effectiveness and crop yields.

Author's Contribution

BA performed experiment and analyzes the data. SJ supervised research and thoroughly revised the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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