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Plant Growth-Promoting Rhizobacteria and Chabazite Zeolite in the growth stimulation and protection of *Sedum palmeri* and *Sedum sieboldii* from aphid and red spider mite attack

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Abstract

Research objective: The aim of this research was to evaluate the interaction that can develop between zeolite and microorganisms with beneficial effects on both the growth of *Sedum palmeri* and *Sedum sieboldii* and in the defence against *Aphis sedii* and *Tetranychus urticae*.

Materials and Methods: The experiments, which started in January 2023, were conducted in the CREA-OF greenhouses in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E). The plants were placed in pots with a diameter of 12, 10 plants per 3 replications, for a total of 30 seedlings per experiment. The Sedum trial included the following theses (irrigated and fertilised): peat 70% + pumice 30% (CTRL); peat 70% + pumice 10% + chabazite natural zeolite 20%; peat 70% + pumice 30% + microorganisms (*Azospirillum brasilense, Pseudomonas kilonensis* and *Bacillus aryabhattai*), 1g/Kg of substrate at the time of substrate preparation; peat 70% + pumice 10% + chabazite natural zeolite 20% and microorganisms (the same as those used in the thesis described above) in the same dosage. A second parallel experiment to control aphids and red spider mite involved: water treatment; treatment for aphids and red spider mite (soft potassium soap and azadirachtin 2.5 ml/l once a week; Kanemite 3 ml/l once a week); treatment for aphids and the alternative red spider mite (micronised zeolite 15g/l and microorganisms 1g/l, once a week). Plant height, leaves and flowers number, vegetative weight, root volume and length, microorganisms in the substrate, number of plants affected by aphids and red spider mite were determined on 15 September 2023.

Results and Discussion: The experiment showed that the use of chabazite zeolite in combination with microorganisms can indeed significantly improve the vegetative and root growth of *Sedum palmeri* and *Sedum sieboldii* plants. Differences in growth between the use of zeolite and microorganisms and the untreated control are evident, with a significant improvement when the two theses are combined for all agronomic parameters analysed. In the substrate analysis, a greater presence of microbial biomass was found in the theses with zeolite, microorganisms and zeolite together with microorganisms than in the control theses, with a greater superiority of microorganisms in the substrate when zeolite and microorganisms are used in combination. The trial with micronised zeolite and microorganisms showed a significant result on the control of aphids and red spider mites, with data comparable to those of treatment with conventional chemical products. Zeolites are used successfully in the cultivation of many crops. Including cereals, vegetables, grapes and other fruits. By enhancing the absorption ability of soil, zeolite enhance the long-term quality of soil by retaining nutrients.

Conclusions: For agricultural production, zeolites must have uniform properties and unique characteristics, such as cation exchange capacity and pH. Especially in agriculture and plant cultivation, zeolites improve their exchange

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capacity with the soil and plants through microbial activity. By lowering the pH of the substrate, microorganisms are able to solubilise the minerals retained by the zeolites, making them more available to the roots. Furthermore, zeolites, when micronised on the leaves, do a good job of protecting plants from insects and fungi, especially when used as a preventive measure. This can also ensure a significant reduction in synthetic chemicals and improve plant protection when these strategies are used in combination.

Keywords: Biocontrol; Zeolites; Plant growth; Microrganisms; Rhizosphere; Succulents

1. Introduction

In this paper, zeolite is defined as diagenised pyroclastic rocks containing a predominant (> 50%) zeolite component (quartz, cristobalite, feldspar, plagioclase) with subordinate amounts of other silicate phases (quartz, cristobalite, feldspar, plagioclase) in place of the generic terms (natural zeolite, sedimentary zeolite, rock with zeolite-rich tuff) used in literature [1,2]. In many European (Slovenia, Czechoslovakia, Hungary, Romania, Bulgaria, Greece) and non-European (Turkey, Iran, Russia, United States, Cuba, Japan, China, Australia) countries, "zeolites" consist mainly of clinoptilolite, which is present in variable quantities (40-60%) in diagenised "acid" tuffs. Various proportions (30-70%) of chabazite and phillipsite are found in Italian "basic" ignimbrites, as well as alkaline-potassium ignimbrites [3]. Several characteristics of zeolites are attributed to their zeolite content:

- High cation exchange capacity (140-210 meq/100 g),
- Reversible dehydration, and
- Structural cryptoporosity [4].

They are also characterized by water retention, mechanical resistance, permeability, and low density due to their lithological nature (micro and macroporosity in texture, lithoid consistency) [5]. Depending on its type and concentration (weight percentage), zeolite exhibits different properties. A volcanic rock's other properties are determined by its nature (tuff, suffice, ignimbrite) and diagenetic process (open, closed, autoclave) [6]. The zeolitic species chabazite and phillipsite have cation exchange capacities of approximately 330-340 meq/100g and are found in microporous and macroporous ignimbrites, while the zeolitic species clinoptilolite occurs in compact tuffs and has cation exchange capacities of 220-230 meq/100g [7]. In contrast to clinoptilolite zeolites widely used elsewhere, the chabazite and phillipsite zeolites popular in Italy show higher CSC and water retention values as well as lower density values [8]. It is crucial to use a large amount of zeolite per square meter both within the root zone and on the soil surface to achieve a qualitative plant growth result [9]. In field conditions, zeolite is usually added at a rate of 2 kg per square metre, while in pot cultivation, it is added at a rate of 20% by weight. It is important for zeolite to be able to exchange cations and to retain water in sandy soils as well as in other situations where water resources are lacking. Plant growth and resistance to biotic and abiotic stresses can be altered by zeolite quality and quantity used in various crops [10].

1.1. Agricultural and environmental aspects of zeolites

A number of laboratory and field experiments have shown that zeolites, especially Italian zeolites containing chabazite, have excellent drainage, water retention, and extraction capacities, as well as a high potassium (K) and a low sodium (Na) content [11]. In this way, fertilizers and irrigation water can be used less, which leads to an increase in agronomic production. Besides reducing irrigation water use, zeolite's permeability and high water retention reduce, respectively, the loss of irrigation and meteoric water due to surface runoff in soils with high clay content (impermeable) and rapid drainage in soils with high sandy content (low water retention) [12]. As a result of the infinitely reversible property of zeolitic water, it can maintain almost constant levels of humidity and temperature at roots by dehydrating (endothermic process) and rehydrating (exothermic process) [13]. A number of experiments were conducted to reduce the adverse effects of high temperatures and droughts [14]. In substrates or soil, zeolite provides excellent protection against cold for ornamental plants, as well as other species, such as vines. Leaf scorch is observed to resume more rapidly at temperatures suitable for cultivation, while excessive levels of salinity in irrigation water are observed to decrease [15]. It has been shown that adding 20% zeolite to Loropetalum sinensis', Aloe, Impatiens, Oleander, Camellia, Crassula growing medium improves plant growth and leaf quality, according to several research studies [16]. Zeolite has been shown by Prisa to reduce (or even eliminate) plant stress caused by excess salts in irrigation water by using it in growing medium [17]. In addition to increasing soil cation exchange capacity (CSC), using less fertilizer also improves soil fertility. It is temporary to remove ammoniacal nitrogen (NH₃) that crops don't need by leaching it into groundwater and by volatilizing it into the atmosphere as greenhouse gases [18]. Besides reducing phosphorus retrogradation (from monocalcium phosphates supplied by fertilizers), this reduces crop assimilation of tricalcium phosphate (Ca₃(PO₄)₂) by reacting with calcium (Ca) in the soil, which is an insoluble phosphate that cannot be assimilated by crops) [19]. Depending on the phenological needs of crops, zeolite releases nitrogen slowly and gradually. Moreover, zeolite absorbs

ethylene produced by ripe fruits, which carries them to plant structures because of its reflective properties [20]. Zeolite also makes it difficult for pathogenic insects to locate ripe fruits. Studies have shown that zeolites attract beneficial microbial colonies, which can enter into symbiosis with plants and enhance water uptake, nutrient availability, and defence by producing secondary metabolites [21-23].

1.2. PGPR: Plant Growth Promoting Rhizobacteria

The rhizosphere is defined as the portion of soil specifically influenced by the plant root system [24,25]. Compared to non-rhizospheric soil, this part of the soil so closely in contact with the root is richer in nutrients, as a result of the accumulation of root exudates, a complex of substances such as amino acids and sugars, which are a source of energy and nutrients for microorganisms [26,27]. The root exudates contain a wide variety of substances from crushed cells and plant tissue, mucilage, volatile substances and soluble exudates released from damaged and intact cells [28]. This condition is reflected in the number and type of bacteria found around the plant roots; although the number of microorganisms in rhizospheric soil is 10 to 100 times higher than in bare, rootless soil, the selective effect of the plant on rhizospheric microbial communities is often well marked and biodiversity is generally reduced [29]. The free-living beneficial bacteria are generally referred to as plant growth-promoting rhizobacteria and, regardless of the mechanisms by which they promote growth, colonise the rhizosphere, the rhizoplane or the interior of the root tissues themselves [30]. Their characteristics are quite diverse and include bacteria active in numerous key processes, such as biological control of phytopathogens or matter cycles [31]. PGPRs belong to several genera of which the main ones are Pseudomonas, Azoarcus, Azospirillum, Rhizobium, Azotobacter, Arthrobacter, Bacillus, Clostridium, Enterobacter, Serratia and others [32-34]. In recent decades, they have been the subject of much research aimed at clarifying the molecular mechanisms underlying their interactions with plants. It is now clear that they can influence the growth and health of many different plant species directly or indirectly. Direct growth promotion involves supplying the plant with compounds synthesised by the same bacteria that colonise its roots, or facilitating, by various mechanisms, the uptake of nutrients from the environment [35,36]. Indirect promotion, on the other hand, occurs when PGPRs reduce or prevent the deleterious effects of one or more phytopathogenic microorganisms through actions such as sequestering iron in the rhizosphere, synthesising antagonistic substances or enzymes that lyse fungal walls, competing on binding sites on the root surface or through inducing systemic resistance. Extensive scientific literature describing the role of PGPRs in sustainable agriculture is available, and numerous reviews or scientific articles clarifying their functions are available [28,29].

Research Objectives

The aim of this research was to evaluate the interaction that can develop between zeolite and microorganisms with beneficial effects on both the growth of *Sedum palmeri* (Figure 1) and *Sedum sieboldii* and in the defence against *Aphis sedii* and *Tetranychus urticae*.



Figure 1 Details of the plants used in the experiment at CREA-OF

2. Material and methods

The experiments, which started in January 2023, were conducted in the CREA-OF greenhouses in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on *Sedum palmeri* and *Sedum sieboldii*.

The plants were placed in pots with a diameter of 12, 10 plants per 3 replications, for a total of 30 seedlings per experiment.

All plants were fertilised with a controlled-release fertiliser (2 kg m⁻³ Osmocote Pro®, 9-12 months with 190 g/kg N, 39 g/kg P, 83 g/kg K) mixed with the growing medium before transplanting. The Sedum trial included the following theses (irrigated and fertilised):

- Peat 70% + pumice 30% (CTRL)
- Peat 70% + pumice 10% + chabazite natural zeolite 20% (ZEOCHAB);
- Peat 70% + pumice 30% + micro-organisms (*Azospirillum brasilense, Pseudomonas kilonensis* and *Bacillus aryabhattai;* 2x109), collection of micro-organisms present in the laboratories of the CREA Horticulture and Floriculture in Pescia, 1g/Kg of substrate at the time of substrate preparation (MICRO);
- Peat 70% + pumice 10% + chabazite natural zeolite 20% and micro-organisms (the same as those used in the thesis described above) in the same dosage (ZEOMICRO).

A second parallel experiment to control aphids and red spider mite involved:

- Water treatment (CTRL);
- Treatment for aphids and red spider mite (soft potassium soap and azadirachtin 2.5 ml/l once a week; Kanemite 3 ml/l once a week) (AFRA);
- Treatment for aphids and the alternative red spider mite (micronised zeolite 15g/l and microorganisms 1g/l, once a week) (ZEOMIC).

The plants were watered once a day and grew for nine months. The plants were drip-irrigated. Irrigation was activated by a timer whose programme was adjusted weekly according to weather conditions and leaching fraction.

Plant height, leaves and flowers number, vegetative weight, root volume and length, microorganisms in the substrate, number of plants affected by aphids and red spider mite were determined on 15 September 2023.

2.1. Methods of analysis

Microbial count: direct determination of the total microbial count by microscopy of the cells contained in a known volume of sample using counting chambers (Thoma chamber). The surface of the slide is etched with a grid of squares, with the area of each square known. Determination of viable microbial load after serial decimal dilutions, spatula seeding (1 ml) and plate counting after incubation;

2.2. Statistics

The experiment was conducted in a randomised complete block design. Collected data were analysed by one-way ANOVA, using the univariate GLM procedure, to assess significant differences ($P \le 0.05$, 0.01 and 0.001) between treatments. Mean values were then separated using the LSD test at multiple intervals (P = 0.05). Statistics and graphs were supported by the programmes Costat (version 6.451) and Excel (Office 2010).

3. Results

The experiment showed that the use of chabazite zeolite in combination with microorganisms can indeed significantly improve the vegetative and root growth of *Sedum palmeri* and *Sedum sieboldii* plants (Table 1 and Table 3). Differences in growth between the use of zeolite and microorganisms and the untreated control are evident, with a significant improvement when the two theses are combined for all agronomic parameters analysed. In particular for plant height, number of leaves, vegetative and root weight (Figure 2 and Figure 3) and root length. In the substrate analysis, a greater presence of microbial biomass was found in the theses with zeolite, microorganisms and zeolite together with microorganisms than in the control theses, with a greater superiority of microorganisms in the substrate when zeolite and microorganisms are used in combination. Previous trials had shown that microbiology can increase the performance of zeolite, which normally occurs alone when zeolite is placed in a substrate, but is accentuated if microorganisms are added on purpose.

The trial with micronised zeolite and microorganisms showed a significant result on the control of aphids and red spider mites, with data comparable to those of treatment with conventional chemical products (Table 2 and Table 4). This is a very interesting aspect that highlights the capabilities of micronised zeolite in controlling insects through repellent mechanisms related to flight difficulty, dehydration, breathing difficulties and light reflection.

Groups	Plant height (cm)	Leaves number (n°)	Flowers number (n°)	Substrate total bacteria (Log CFU/g soil)	Vegetative weight (g)	Roots volume (cm ³)	Roots length (cm)
CTRL	25.40 d	22.42 c	6.83 c	1.29 d	28.44 c	18.54 c	3.67 d
ZEOCHAB	26.86 b	26.61 b	8.22 b	1.92 c	29.43 b	19.47 b	4.26 c
MICRO	26.67 c	27.64 b	8.21 b	2.38 b	29.64 b	19.35 b	4.58 b
ZEOMICRO	25.40 d	31.41 a	11.21 a	3.27 a	32.91 a	22.14 a	5.73 a
ANOVA	***	***	***	***	***	***	***

Table 1 Evaluation of the use of different zeolites and microorganisms on Sedum palmeri

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05).Legend: (CTRL) peat 70% + pumice 30%; (ZEOCHAB) peat 70% + pumice 10% + chabazite zeolite 20% (MICRO) peat 70% + pumice 30% + micro-organisms; (ZEOMICRO) peat 70% + pumice 10% + chabazite zeolite 20% and micro-organisms.

Table 2 Evaluation of the use of different zeolites and microorganisms as a protective treatment against aphids and redspider mite on Sedum palmeri

Groups	Aphis sedii (n°)	Tetranychus urticae (n°)
CTRL	11.00 a	5.60 a
AFRA	2.20 b	2.60 b
ZEOMIC	1.40 b	1.50 c
ANOVA	***	***

One-way ANOVA; n.s. – non significant; *,*,*** – significant at P \leq 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05).Legend: (CTRL) water treatment; (AFRA) soft potassium soap and azadirachtin 2.5 ml/l once a week; Kanemite 3 ml/l once a week; (ZEOMIC) micronised zeolite 15g/l and microorganisms 1g/l

Table 3 Evaluation of the use of different zeolites and microorganisms on Sedum sieboldii

Groups	Plant height (cm)	Leaves number (n°)	Flowers number (n°)	Substrate total bacteria (Log CFU/g soil)	Vegetative weight (g)	Roots volume (cm ³)	Roots length (cm)
CTRL	15.33 c	16.00 c	7.00 c	1.73 d	22.87 c	16.79 d	3.69 d
ZEOCHAB	16.37 b	18.21 b	8.84 b	2.22 c	23.68 b	17.68 c	4.47 c
MICRO	16.53 b	18.22 b	9.23 b	2.93 b	23.77 b	18.07 b	4.80 b
ZEOMICRO	17.63 a	22.00 a	11.21 a	3.16 a	24.72 a	21.34 a	5.14 a
ANOVA	***	***	***	***	***	***	***

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05).Legend: (CTRL) peat 70% + pumice 30%; (ZEOCHAB) peat 70% + pumice 10% + chabazite natural zeolite 20 (MICRO) peat 70% + pumice 30% + micro-organisms; (ZEOMICRO) peat 70% + pumice 10% + chabazite natural zeolite 20% and micro-organisms.

Table 4 Evaluation of the use of different zeolites and microorganisms as a protective treatment against aphids and redspider mite on Sedum sieboldii

Groups	Aphis sedii (n°)	Tetranychus urticae (n°)		
CTRL	9.60 a	6.40 a		
AFRA	2.80 b	2.70 b		
ZEOMIC	1.80 b	2.00 b		
ANOVA	***	***		

One-way ANOVA; n.s. – non significant; *, *, *, *** – significant at $P \le 0.05$, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05).Legend: (CTRL) water treatment; (AFRA) soft potassium soap and azadirachtin 2.5 ml/l once a week; Kanemite 3 ml/l once a week; (ZEOMIC) micronised zeolite 15g/l and microorganisms 1g/l



Figure 2 Comparison between the thesis with peat 70% + pumice 30% (CTRL), peat 70% + pumice 10% + chabazite natural zeolite 20 (ZEOCHAB), peat 70% + pumice 30% + micro-organisms(MICRO) and peat 70% + pumice 10% + chabazite natural zeolite 20% and micro-organisms (ZEOMICRO) on the vegetative and radical growth of *Sedum palmeri*



Figure 3 Comparison between the thesis with chabazite natural zeolite 20% and micro-organisms (ZEOMICRO) and peat 70% + pumice 30% (CTRL) on the vegetative growth of *Sedum sieboldii*

4. Discussion

Fredrich Cronstet, a Swedish mineralogist, discovered zeolite as a mineral in 1756 [37-39]. This mineral is also known as "boiling stones" since it is capable of frothing at temperatures up to 200°C. The zeolites have been rediscovered since the 1950s, and commercial production and use have begun around the world [40]. For two hundred years following their discovery, zeolites were considered volcanic rock minerals [41]. Different groups have reported more than forty types of zeolites to date. In addition, more than 150 zeolites have been synthesized, including analcime (sometimes called analcite), clinoptilolite, erionit, chabazite, mordenite, and philipsite. These naturally occurring and synthetic minerals are widely used in commercial applications because of their adsorption, ion exchange, molecular sieve, and catalytic properties [42-44]. Providing sufficient quantities of zeolites that have uniform characteristics as well as unique properties (cation exchange capacity, pH, and B content) for application and commercial processing requires extensive research [45]. To enhance the use of zeolites, researchers are conducting research. Zeolites without well defined chemical characteristics may cause severe problems in their application because they have cage-like structures, have similar properties, or are associated with zeolites. In addition to agriculture, zeolites are used for ion exchange, filtering, odour removal, chemical sieving, water softening, and gas absorption [46]. Production, soil stabilization, building materials, anticorrosive paint components, defluorinating industrial wastes, desulphurizing flue gas, removing methylene blue and mercury, recovering copper from phosphate fixation, neutralizing acid waste, cleaning sewers, and removing heavy metals and ammonium ions are among them [47].

Zeolites are composed of pores and cornersharing aluminosilicate (AlO₄ and SiO₄) tetrahedrons, joined into three dimensional frameworks. The pore structure is characterized by cages approximately 12Å in diameter, which are interlinked through channels about 8Å in diameter, composed of rings of 12 linked tetrahedrons. As a result of their interconnections, pores form long, wide channels of varying sizes [48]. These channels allow ions and molecules to move into and out of the structure, forming honeycombs or cages. Aluminium has a negative charge, which is balanced by positively charged cations. There are large voids or cages within zeolite, which resemble honeycombs or cages. Using zeolite in agriculture can help improve fertilizer efficiency, leading to a better crop yield. According to some authors, 48 pounds of zeolite per acre can increase yields [49]. With 2 to 8 kg/tree, this mineral contributes to better new orchard establishment. Zeolite is used successfully for many crops. Among them are cereals, vegetables, grapes, and other fruits. Through their ability to enhance soil absorption, zeolites enhance the long-term quality of soil by retaining nutrients. In addition to nitrogen (N) and potassium (K), it also includes calcium, magnesium, and microelements. which are important plant nutrients. Thus, using zeolite can reduce the amount of N and K fertilizer needed to achieve the same yield, prolong their activity, or boost their efficiency [50]. Sandy soils, which cannot retain nutrients, lose large quantities of fertilizers through leaching. Therefore, by reducing nutrient loss, zeolite will assist plants in growing and developing. In zeolite, crystalline structures can hold up to 60% of their weight in water, but water molecules in pores can easily be evaporated or reabsorbed without damaging the structure. In addition to providing permanent moisture during dry times, zeolites also aid in rapid rewetting during irrigation and improve the lateral spread of water. It is possible for zeolites to absorb pesticides and reduce irrigation water needs. Besides positive cations such as sodium, potassium, barium, and calcium, negative-charged zeolites can trap positively charged groups such as water and ammonia. In addition to attracting carbonate and nitrate ions, zeolites are also able to absorb water because of their negative charge. Soil alkali metallic cations and alkali metallic cations can also be attracted by zeolites. As a result of their weak attraction, zeolites can replace absorbed cations, making them good ion exchangers. Zeolite improves nutrient retention in soil by remaining in it permanently, unlike other soil amendments (such as lime). In addition to reducing water and fertilizer costs, its addition to soil will retain beneficial nutrients in the root zone. Zeolite also keeps soil active for a long time because of its porous structure, which keeps it aerated and moist. When zeolite is used with fertilizers, soil pH levels are buffered, reducing lime application. Due to its marginal acidity, zeolite is not acidic but marginally alkaline [51]. Use of this mineral can result in very high irrigation and maintenance costs for golf courses and sports fields [52,53]. A significant improvement in vegetative and root growth was observed in this study with the addition of chabazite zeolite and microorganisms to Sedum palmeri and Sedum sieboldii, with obvious effects on the microbial biomass of the added substrate as well. Compared to conventional chemical treatment of these theses, micronised zeolite and microorganisms enhanced plant defense. Prior to use, zeolite needs to be pre-inoculated with microbial strains

5. Conclusion

Due to its high absorption rate, cation exchange, catalysis and dehydration capacity, chabazite is the most common zeolite for agricultural applications. Therefore, zeolite fertilisers are used to improve plant growth. In addition, they can be used as molecular sieves or filters and retain nitrogen in the manure and sludge produced. For agricultural production, zeolites must have uniform properties and unique characteristics, such as cation exchange capacity and pH.

Especially in agriculture and plant cultivation, zeolites improve their exchange capacity with the soil and plants through microbial activity. By lowering the pH of the substrate, microorganisms are able to solubilise the minerals retained by the zeolites, making them more available to the roots. Furthermore, zeolites, when micronised on the leaves, do a good job of protecting plants from insects and fungi, especially when used as a preventive measure. This can also ensure a significant reduction in synthetic chemicals and improve plant protection when these strategies are used in combination.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares no conflict of interest.

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