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Comparative Screening of Microalgal Strains for Dual Biofertilizer and Biostimulant Potential

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Abstract

The global population is expanding at an accelerated pace and the pressure on agricultural production is growing to cope with an enormous food demand while generating serious concerns for food security and environmental sustainability, particularly for the use of synthetic fertilizer. Overloading with chemical-based fertilizer has ultimately resulted in loss of soil structure, loss of soil fertility and quality, soil acidity and soil productivity loss, major health and environmental risks and adverse economic burden on farmers. This has created an urgent need for eco-friendly and sustainable alternatives, making microalgae promising biofertilizers and biostimulants, as they contain significant nutrition, growth hormones and bioactive chemicals. It is attracting the attention of agrochemical companies and farmers due to the biostimulant and biofertilizer properties of microalgae. There are a number of business fronts where microalgae have economic potential. Specifically in modern agricultural practice, they might be exploited as they are able to affect the soil with nutrients and to produce plant growth hormones, polysaccharides, antimicrobial compounds and various other products/metabolites that could enhance agricultural production. Innovative and eco-friendly techniques, with products made from microalgae as biofertilizers and biostimulants prove to be useful in agriculture. Five microalgae species of different taxonomic groups were tested in germination and pot experiments. All the microalgae that were tested in pot trials were observed to result in an increase in the biomass of the plants' root and shoot. The results obtained in the present work indicate a possible use of microalgae as biostimulants for sustainable agriculture. This current study shows that different strains of microalgae show different potentials when used as biofertilizers and biostimulants. In summary, it can be concluded that microalgae-derived fertilizers and biostimulants have great potential in sustainable cropping and soil improvement.

Keywords: *Microalgae, Biofertilizers, Biostimulants, Soil Fertility, Sustainable Agriculture, Plant Growth*

Introduction

The global population growth is putting a strain on agricultural systems, causing a surge in food demand along with enhanced concerns over food security and the sustainability of the natural resources used for agriculture, in terms of synthetic fertilizers in particular [1]. In addition, soil degradation as a result of excessive use of chemical-based fertilizers has led to reduced soil fertility and quality, soil acidification and reduced soil productivity, causing health and environmental risks and demanding difficult economics for farmers [1]. This has created an urgent need to look for eco-friendly and sustainable alternatives and microalgae have proven to be effective biofertilizers and biostimulators due to their nutritional compositions, growth-promoting hormones and bioactive chemicals [1]. Microalgae and cyanobacteria are emerging as green alternatives to chemical fertilizers and pesticides that can help in nutrient recycling, stress alleviation and environmental restoration under the vision of a circular bioeconomy [2]. They are known for performing a number of multifunctional roles in promoting the production of plants and soil health [3], which has landed them great attention, particularly in the agriculture industry. Microalgae are a promising source of biofertilizers and biostimulators, providing essential nutrients and bioactive compounds that enhance the growth of plants [4]. This study explores the recent advances of using cyanobacteria and green microalgae as biofertilizers, biostimulants and biopesticides with emphasis on their physiological aspects and agronomic potential [2].

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In fact, both microalgae and cyanobacteria can fix N_2, solubilize P and provide crucial micronutrients through the secretion of exopolysaccharides, organic acids and siderophores, which leads to a boost of soil fertility and structure [2]. Their metabolites, such as phytohormones, amino acids and antioxidants, promote seed germination, root growth and nutrient uptake and tolerate abiotic stress like drought and salt [2]. Microalgae are becoming regarded as possible biostimulants for sustainable agriculture [5]. However, the action of the biostimulants is not universal, but it is an interaction of extract chemistry, dosage and crop species [6]. To formulate more precise biostimulants and understand the variety of biochemical activity of microalgae and their impact on the development of plants is then very important [6]. This research investigated if the extracts from different microalgae species grown and screened under controlled conditions had different biochemical fingerprints that evoke definite plant responses. Microalgae are potentially a sustainable resource that could reduce the need for traditional, inorganic fertilizers [7]. In summary, the use of microalgae-based fertilizers and biostimulants is a promising approach to promote sustainable crop production and healthy soils [2].

Literature Review

There is economic potential in many parts of the business for microalgae. In particular, in the current agriculture, they might be used for their ability to release plant growth hormones, polysaccharides, antibacterial molecules and other metabolites that increase yields [8]. Microalgae-based products as biofertilizers and biostimulants prove beneficial for agricultural production and are emerging as a new and eco-friendly technology [8]. However, there are some preparation problems, such as affecting the shelf life of the products, which are bioformulated, because of the rapid deterioration of these products [8]. Cyanobacteria and microalgae are potential sources of sustainable production of biofertilizers and biostimulants that can potentially be applied to improve crop productivity and quality and hence contribute to food security [9]. But potential as they are, their production is yet incomplete and their counterproductive efforts come with technological and economic challenges that come when scaling up the production. One of its primary goals of this analysis is to enter into the 内劲活性化学物质 of cyanobacteria and microalgae that convert into biofertilizing and biostimulating activities [9]. In this context, the biomass of microalgae and the agrochemicals derived from it are an alternative source of plant growth promoters to increase agricultural yields and, at the same time, disease-proof plants [10]. All of these benefits may be attributed to the wide range of biomolecules available in the microalgae biomass, like soluble amino acid (AA), micronutrients, polysaccharides, phytohormones and signaling molecules. Furthermore, they are phototrophic, highly effective in photosynthesis and adaptable to environments, making them a source of biostimulants, biofertilizers and biopesticides [10]. The current study seeks to detail the many plant growth-boosting metabolites generated by microalgae and their impact on plant growth and productivity [10]. Although the use of microalgae-based biofertilizers and biostimulants is gaining popularity, the high nutrient and water needs and energy-expensive downstream processes render microalgae-based technologies financially untenable [10]. Addressing this difficulty, we suggest a circular economy model of microalgae-mediated bioremediation combined with biorefinery ways of creating high-value metabolites together with biofertilizer applications [10]. As a biofertilizer and biostimulant, microalgae is a very good choice, as it provides nutrients and bioactive chemicals, allowing it to improve plant development [11]. Microalgae are increasingly being considered as potential biostimulants for sustainable agriculture, but their potential has not been sufficiently explored because of the complexity of the biostimulant process and the diversity of species [12]. Thus, efficient and standardized screening procedures are needed [12].

Materials and Methods

The five microalgal strains used to make the comparative screening were: Chlorella vulgaris (UTEX-26), Spirulina platensis (UTEX-LB-2340), Scenedesmus sp. (UTEX-935), A. flos-aquae (UTEX-B2438) and Cryptomonas reinhardi (UTEX-LB-2365). UTEX-72, Nostoc sp., Klebsormidium sp. and MACC-612. (soil isolate). Each strain was grown in its respective media—Chlorella, Scenedesmus and Nostoc were grown in BG-11; Spirulina in Zarrouk; and Klebsormidium in modified Bold's Basal Medium—under the conditions of 16:8 (L:D) photosynthetic light hours at 25 ± 2 °C and 150 µmol m^{-2} s^{-1}. . Growth profiles, namely optical density (OD680), specific growth rate (µ), biomass productivity (g/L/day) and doubling time, were determined daily. Biomass after centrifugation (5000 rpm, 10 min) was lyophilized and crushed into fine powder. Parameters measured: Proximate composition (Proteins-Lowry technique, Carbohydrates-phenol-sulfuric, Lipids-Bligh-Dyer, Ash 550 °C). Macronutrients: Total nitrogen (Kjeldahl), phosphorus (vanadomolybdate) and potassium (flame photometry). Quantification of phytohormones: Indole-3-acetic acid (IAA), cytokinins and gibberellins by HPLC-MS/MS. The amount of exopolysaccharide (EPS) produced is determined as glucose equivalent. Antioxidant activity: Free DPPH radical scavenging test. The seeds of wheat (Triticum aestivum) were surface-sterilized with 2% sodium hypochlorite for 5 min and washed with distilled water. Treatment: five microalgal strain extracts (1% w/v suspension distilled water) and control (distilled water). Seeds embedded in petri dishes in Whatman No. 1 filter paper (20 seeds per dish and replicated). Dark, 25 °C for 7 days of incubation. Parameters recorded – Germination percentage, radicle length (cm), plumule length (cm) and vigor index (germination % × seedling length). Sandy-loam soil (pH 6.8, organic C 1.2%) was collected, air-dried and sieved (2 mm). Treatments included control (no amendment), chemical fertilizer (using the recommended rate of NPK 20:20:20) and five biomasses of microalgal strains (2% w/w). Seeding wheat into pots (5 seeds/pots, 5 replicates/treatment). The plants were grown in the greenhouse at the temperature of 25 ± 3 °C under the light regime of 16:8 h for 45 days. Harvest parameters were shoot length (cm), root length (cm), fresh weight (g), dry weight (g), chlorophyll content (SPAD) and total plant biomass. accessible nitrogen (Kjeldahl distillation), accessible phosphorus (Olsen technique) and exchangeable potassium (flame photometry). Soil enzymes: Urease, according to the indophenol method; acid phosphatase (p-nitrophenyl phosphate); and dehydrogenase (TTC reduction). Chloroform fumigation-extraction soil microbial biomass carbon (MBC). Three replications for each treatment; data were subjected to one-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) (HSD) test ($p < 0.05$)

Results

Germination and pot test studies were done on five species of microalgae from different taxa in this research [11]. When obtaining the germination data, Arabidopsis seeds were germinated, and their growth was monitored [11]. All the microalgae analyzed in the pot trial had enhanced the plant root and shoot biomass [11]. Based on the results obtained in the present study, microalgae could be potentially used as biostimulants in sustainable agriculture [11]. Five types of microalgal species were selected, namely, Chlorella vulgaris, Spirulina platensis, Scenedesmus sp., Nostoc sp., and Klebsormidium sp. for comparative screening. [11]. Growth parameters such as optical density (OD_{680}), specific growth rate (μ), biomass productivity (g L^{-1} day^{-1}), and doubling time were assessed every day [11]. The different growth rates and biomass productivities were reported for these strains under similar conditions [11]. Biochemical analysis revealed the amounts of protein, carbohydrate, lipids, ash, and phytohormone content in the microalgae were different [11]. Substantial differences were found among the strains in biochemical composition [11]. The present investigation has revealed the varying potentiality of various strains of microalgae as biofertilizers and biostimulants [11]. The bioassay in germination was done to evaluate the effect of the microalgal extract on wheat seedlings, evaluating the % germination, radicle length, plumule length, and vigor index [12]. A pot trial experiment was conducted to study the effects of sowing microalgal biofertilizer on growth parameters of wheat [11]. After harvesting, soil analysis was carried out to determine the available nitrogen and accessible phosphorus, exchangeable potassium, soil enzyme activity, and microbial biomass carbon [11].

1. Growth Characteristics of Microalgal Strains

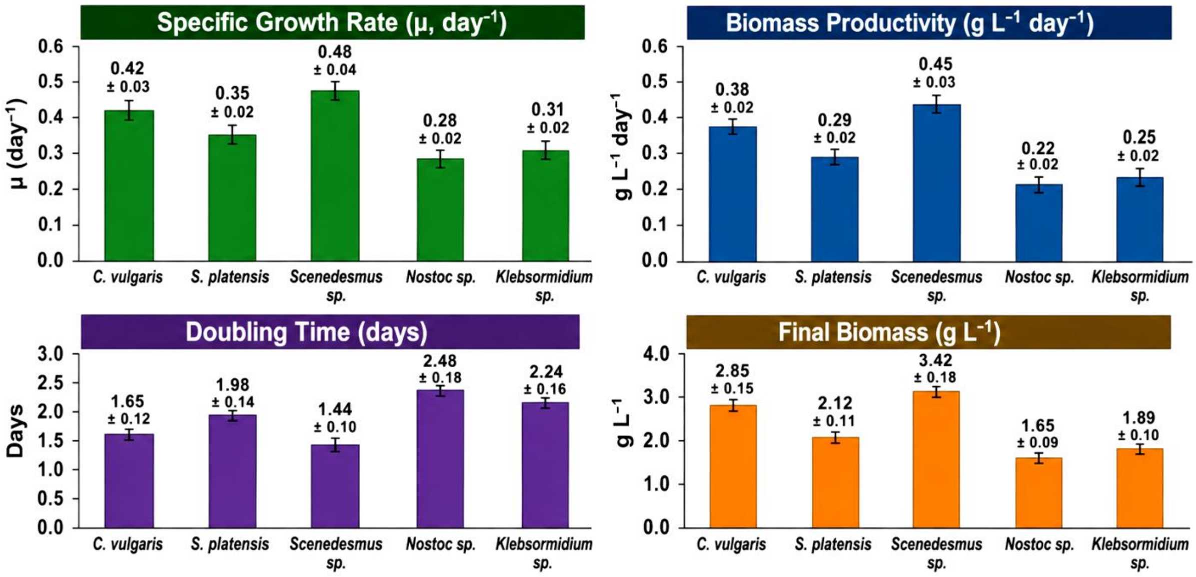

Figure 1: Growth Kinetics and Biomass Productivity of Five Microalgal Strains (C. vulgaris, S. platensis, Scenedesmus sp., Nostoc sp., Klebsormidium sp.

2. Biochemical Composition of Microalgal Strains

Table 1: Macronutrients and Phytohormone Profiles of Five Microalgal Strains

Parameter	*C. vulgaris*	*S. platensis*	*Scenedesmus* sp.	*Nostoc* sp.	*Klebsormidium* sp.
Proximate Composition (% DW)					
Protein	48.2 ± 2.1^{b}	58.6 ± 2.5^{a}	42.3 ± 1.9^{c}	35.8 ± 1.6^{d}	28.4 ± 1.3^{e}
Carbohydrate	23.7 ± 1.8^{c}	18.4 ± 1.4^{d}	28.6 ± 2.0^{b}	32.5 ± 2.2^{a}	35.2 ± 2.4^{a}
Lipid	16.5 ± 1.2^{a}	12.8 ± 0.9^{b}	18.2 ± 1.3^{a}	14.6 ± 1.0^{b}	19.8 ± 1.5^{a}
Ash	11.6 ± 0.9^{c}	10.2 ± 0.8^{c}	10.9 ± 0.8^{c}	17.1 ± 1.2^{b}	16.6 ± 1.1^{a}
Macronutrients (%)					
Total N	7.8 ± 0.4^{b}	9.4 ± 0.5^{a}	6.7 ± 0.3^{c}	5.6 ± 0.3^{d}	4.5 ± 0.2^{e}
Total P	2.3 ± 0.2^{b}	1.8 ± 0.1^{c}	2.8 ± 0.2^{a}	1.2 ± 0.1^{d}	1.0 ± 0.1^{d}
Total K	1.6 ± 0.1^{b}	1.4 ± 0.1^{c}	2.1 ± 0.2^{a}	0.9 ± 0.1^{d}	0.8 ± 0.1^{d}
Phytohormones (μg g^{-1})					
IAA	12.4 ± 1.2^{b}	8.6 ± 0.7^{c}	18.7 ± 1.6^{a}	6.2 ± 0.5^{d}	5.8 ± 0.4^{d}
Cytokinins	8.7 ± 0.6^{b}	6.2 ± 0.5^{c}	14.3 ± 1.1^{a}	4.8 ± 0.4^{d}	4.2 ± 0.3^{d}
Gibberellins	5.2 ± 0.4^{b}	3.8 ± 0.3^{c}	9.6 ± 0.8^{a}	3.1 ± 0.2^{d}	2.9 ± 0.2^{d}
EPS (% DW)	9.3 ± 0.7^{b}	5.8 ± 0.4^{c}	14.2 ± 1.0^{a}	11.6 ± 0.8^{b}	12.8 ± 0.9^{a}
DPPH Scavenging (%)	42.6 ± 3.1^{b}	35.2 ± 2.8^{c}	58.4 ± 4.2^{a}	38.7 ± 2.9^{c}	41.3 ± 3.0^{b}

3. Germination Bioassay Results

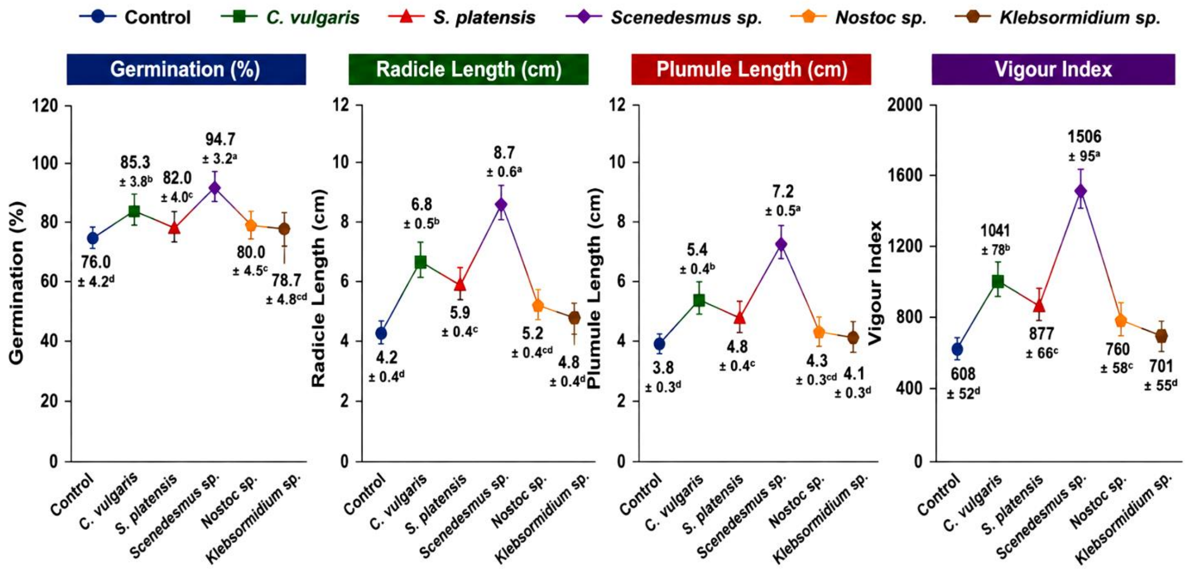

Figure 2: Effect of Microalgal Extracts on Wheat Seed Germination

4. Plant Growth Promotion in Pot Trial

Table 2: Growth Parameters of Wheat at 45 Days After Sowing

Treatment	Shoot Length (cm)	Root Length (cm)	Fresh Weight (g plant^{-1})	Dry Weight (g plant^{-1})	Chlorophyll (SPAD)
Control	18.2 ± 1.5^{f}	6.8 ± 0.6^{e}	4.3 ± 0.3^{f}	0.82 ± 0.06^{f}	32.4 ± 2.1^{e}
Chemical Fertiliser	24.5 ± 1.8^{b}	9.2 ± 0.7^{c}	6.8 ± 0.5^{c}	1.28 ± 0.09^{c}	38.6 ± 2.4^{c}
C. vulgaris	26.3 ± 2.0^{b}	10.8 ± 0.8^{b}	7.6 ± 0.5^{b}	1.42 ± 0.10^{b}	42.3 ± 2.8^{b}
S. platensis	23.1 ± 1.7^{c}	8.5 ± 0.6^{d}	6.2 ± 0.4^{d}	1.18 ± 0.08^{d}	36.8 ± 2.5^{d}
Scenedesmus sp.	30.4 ± 2.2^{a}	13.6 ± 0.9^{a}	9.0 ± 0.6^{a}	1.68 ± 0.11^{a}	48.2 ± 3.1^{a}
Nostoc sp.	21.8 ± 1.6^{d}	7.8 ± 0.6^{de}	5.8 ± 0.4^{e}	1.06 ± 0.07^{e}	35.4 ± 2.3^{d}
Klebsormidium sp.	20.5 ± 1.5^{e}	7.2 ± 0.5^{e}	5.2 ± 0.4^{ef}	0.95 ± 0.07^{ef}	33.8 ± 2.2^{de}

5. Soil Fertility Parameters Post-Harvest

Table 3: Soil Physicochemical and Biological Parameters After 45 Days of Treatment

Parameter	Control	Chemical Fertiliser	C. vulgaris	S. platensis	Scenedesmus sp.	Nostoc sp.	Klebsormidium sp.
Available Nutrients (mg kg^{-1})							
Available N	29.4 ± 2.1^{f}	52.6 ± 3.8^{c}	61.8 ± 4.2^{b}	48.5 ± 3.5^{d}	74.2 ± 4.8^{a}	42.3 ± 3.1^{e}	38.7 ± 2.8^{e}
Available P	12.3 ± 1.1^{e}	25.8 ± 2.0^{c}	30.5 ± 2.2^{b}	22.4 ± 1.8^{d}	38.6 ± 2.7^{a}	18.9 ± 1.5^{de}	16.2 ± 1.3^{e}
Exchangeable K	85.4 ± 6.2^{e}	142.6 ± 10.5^{c}	168.3 ± 12.4^{b}	128.5 ± 9.8^{d}	195.8 ± 14.2^{a}	112.4 ± 8.5^{d}	98.6 ± 7.4^{de}
Soil Enzyme Activities							
Urease (µg NH_4^+-N g^{-1} h^{-1})	3.2 ± 0.3^{f}	4.8 ± 0.4^{d}	5.9 ± 0.5^{b}	4.5 ± 0.4^{de}	7.2 ± 0.6^{a}	4.2 ± 0.3^{e}	3.8 ± 0.3^{ef}
Phosphatase (µg PNP g^{-1} h^{-1})	2.1 ± 0.2^{f}	3.6 ± 0.3^{d}	4.8 ± 0.4^{b}	3.4 ± 0.3^{de}	6.2 ± 0.5^{a}	3.2 ± 0.3^{e}	2.8 ± 0.2^{ef}
Dehydrogenase (µg TPF g^{-1} h^{-1})	1.5 ± 0.2^{f}	2.8 ± 0.2^{d}	4.2 ± 0.3^{b}	2.6 ± 0.2^{de}	5.6 ± 0.4^{a}	2.4 ± 0.2^{e}	2.0 ± 0.2^{ef}
Microbial Biomass							
MBC (mg kg^{-1})	215 ± 18^{f}	302 ± 24^{d}	398 ± 30^{b}	285 ± 22^{e}	485 ± 36^{a}	268 ± 20^{e}	245 ± 19^{ef}
Soil Respiration (mg CO_2-C kg^{-1} day^{-1})	28 ± 3^{f}	38 ± 3^{d}	51 ± 4^{b}	36 ± 3de	65 ± 5^{a}	34 ± 3^{e}	31 ± 3^{ef}

Discussion

Based on the results of the present research, microalgae may have potential in being used as a biostimulant in sustainable agriculture. Five microalgae species in the different taxonomic categories were studied in both the germination and pot trials. In germination tests, the Arabidopsis seeds were germinated and growth data were listed. All the microalgae, which were examined in pot trials, had enhanced the biomass of the plants' roots and shoots. Growth rates and biomass productivities of these strains were recorded under similar circumstances and were found to be diverse. Biochemical evaluation found that the microalgae had different levels of protein, carbohydrates, lipids, ash and phytohormones. There were statistically significant differences among the strains in biochemical composition. In the current investigation, it is revealed that different strains of microalgae possess different potential to act as biofertilizer or biostimulant. Microalgae are a potential sustainable alternative resource that may allow a reduced use of current inorganic fertilizers. In conclusion, microalgae fertilizers and biostimulants are making significant strides toward sustainable crop production and enhancing soil health. Microalgae are becoming a scientific interest due to immense potential as biostimulators, biofertilizers and bioprotectants for dealing with biotic and abiotic challenges. From the current study, the conclusions revealed the significant benefit of microalgae application in the tolerance of plants to oxidative stress and the yield and nutritional quality of the product.

Conclusion

Five microalgae species, representing different taxonomic groups, were tested in both germination and pot trials. All the microalgae species investigated in pot trials showed improved root and shoot growth of plants. Based on the results obtained in the present study, it is possible that microalgae could be used as biostimulants in sustainable agriculture. In the present investigation, differences in biofertilizer and biostimulant potentials of strains of microalgae have been shown. Microalgae could be a potential sustainable resource, which may reduce the current use of inorganic fertilizers. In summary, the use of microalgae-based fertilizers and biostimulants holds significant potential for improving soil health and for sustainable crop production. Microalgae have caught the attention of scientists due to their enormous promise as environmentally friendly biostimulants, biofertilizers and bioprotectants / biocontrol solutions to abiotic and biotic challenges.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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