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Research article

Productivity, biological efficiency and bromatological composition of *Pleurotus sajor-caju* growth on different substrates in Brazil

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Abstract

Pleurotus is one of the most important edible mushroom genera in Brazil, specifically *P. ostreatus*, *P. sajor-caju* and *P. ostreatusroseus*. With the increasing culinary use of mushrooms, this research cultivated *P. sajor-caju* on different substrates and evaluated the fresh mass, productivity, biological efficiency and bromatological composition. Cultivation using elephant grass produced 232.40 g of fresh mass (23.24 g productivity); however, better biological efficiency (106.60%) was achieved using rice straw. Higher humidity levels (91.41%) and ether extract (5.65%) were observed in mushrooms produced with elephant grass plus sugarcane bagasse. The bromatological analysis indicated 42.13% crude protein and 7.97% ash in the mushrooms produced with sugarcane bagasse. Mushrooms from the mixed elephant grass and rice straw had 58.66% carbohydrates and only 8.69% crude fiber, while the rice straw experiments had 31.33% crude fiber. These results indicated that using elephant grass resulted in higher values of fresh mass, productivity and biological efficiency for the cultivation of *P. sajor-caju* than the other treatments. The study also indicated the benefits of the use of agricultural wastes for the cultivation of mushrooms, as well as the variability of the nutritional and physicochemical composition thereof for different substrates.

Introduction

The demand and the quality of food have driven several forms of research including the development of techniques for mushroom cultivation in the agro-industrial area aiming to reduce production costs and the price to consumers and to increase global consumption. In Brazil, data on mushroom production is vague, with some anecdotal data showing that the consumption of mushrooms in the country is much higher than from local production, mainly due to the high costs related to production. The genus *Pleurotus*, is widely distributed worldwide and is found in tropical and subtropical forests and is grown artificially for commercial purposes (Mohamed et al., 2014). There are many edible species in this genus such as *Pleurotus ostreatus*, *P. pulmonarius*, *P. sajor-caju* and *P. roseus*, among others (Khan and Tania, 2012). Adebayo et al. (2018) found antioxidant and antibacterial activities in bioactive compounds of *Pleurotus* species, as demonstrated by the interest in the incorporation of *P. sajor-caju* power in food products, providing a novel source rich in dietary fiber (Ng et al., 2017).

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World production of mushrooms was estimated at over 10 million t in 2016 (Food and Agriculture Organization, 2018), and the production of edible mushrooms on natural substrates involves laboratory procedures to obtain strains and produce the inoculants, and in cultivation ranging from inoculation to processing (Zhang et al., 2014). *Pleurotus* spp. produces many lignocellulosic enzymes, which facilitate the degrading of the lignin and cellulose of the wood and other substrates of vegetable origin used for cultivation. The fungi secrete enzymes during their development, degrading compounds to obtain carbon, nitrogen, sulfur and other nutrients needed for their growth (Phan and Sabaratnam, 2012).

Often, the substrates used for mushroom cultivation are nutritionally poor, thus requiring the addition of energetic sources. Sugarcane bagasse, for example, needs supplementation to allow shiitake mushroom cultivation (Nunes et al., 2012). Thus, research on the use of agro-industrial wastes must be done specifically for each situation, in order to select those that result in higher productivity of mushrooms. Rio Grande do Sul - Brazil, is a leading Brazilian state in rice production and processing, especially the southern half of the state, generating a considerable amount of wastes such as straw, rice husks and impurities. Since commercialization of edible mushrooms is already a well-established economic activity, value can be added to these agricultural wastes by using them in the production of protein in the form of fungal biomass (Fonseca et al. 2014).

Patil et al. (2010) described a series of agricultural and agro-industrial wastes that can be recycled in edible mushroom cultivation including soybean, wheat straw, paddy and their combinations. As these materials are agribusiness waste, recycling is desirable ecologically and economically. The purpose of this study was to evaluate the fresh mass, productivity, biological efficiency and bromatological composition of *Pleurotus sajor-caju* (PSC96/03) grown on three main substrates: rice straw, elephant grass and pasteurized sugarcane bagasse.

Materials and Methods

Mushroom strain

The experiment was performed at the Experimental Mycology Laboratory (LEMICO), Department of Microbiology and Parasitology (DEMP), Institute of Biology (IB) of the Universidade Federal de Pelotas (UFPel), Rio Grande do Sul, Brazil. This work used the species *P. sajor-caju* (strain PSC96/03) originating from the Mushrooms Module FCA/UNESP/Botucatu/SP, deposited in the mycology collection LEMICO/DEMP/IB/UFPel. The strain was recovered and multiplied as described in the general methodology below.

Spawn production

Spawn were prepared using rice grains with hulls (*Oryza sativa* L.) previously boiled for 15 min added with 1% gypsum (volume per volume). Then, they were stored in glass flasks (8.6 cm × 14 cm) which were sealed with aluminum foil and plastic wrap and autoclaved at 121°C (1 atmosphere) for 45 min. After sterilization and cooling, the bottles were inoculated with 10 mm diameter culture disks from each strain. Then, they were incubated at 28°C until the grains had been colonized by the fungus, which were used as the inoculum (spawn)**.**

Substrate preparation

The substrates used were sugarcane bagasse (*Saccharum officinarum*), rice straw (*Oryza sativa*), and elephant grass (*Pennisetum purpureum*), which were dried, ground or chopped. The substrates were pre-moisturized for 24 hr, then placed in high-density polypropylene bags and subjected to severe pasteurization at 100ºC for 30 min. The treatments were: T1, elephant grass (100%); T2, sugarcane bagasse (100%); T3, rice straw (100%); T4, mixed elephant grass (50%) and sugarcane bagasse (50%); T5, mixed elephant grass (50%) and rice straw (50%); T6, mixed sugarcane bagasse (50%) and rice straw (50%); and T7, mixed elephant grass (33%), sugarcane bagasse (33%) and rice straw (33%).

Mushroom production

After cooling the substrates at 30°C, they were inoculated with 3% spawn (weight per weight) of *P. sajor-caju* (strain PSC96/03). For this purpose, the substrates were transferred to plastic trays and homogenized with the inoculum. The mixture was placed into plastic bags, each containing 1 kg of wet substrate. The bags were placed on shelves at room temperature (25–28ºC) during the colonization phase. After completing colonization, the bags were transferred to a fruiting chamber where the temperature and relative humidity were monitored and maintained in the ranges 23–28ºC and 75–90%, respectively. The mushrooms were collected manually and quantified in each flow (a flow was considered from the emergence of mushrooms until the fruiting stopped and the non-fruiting interval started). To stimulate fruiting, the bags were immersed in water for 24 hr. In the interval phase, the humidity was reduced to environmental conditions while during the fruiting phase it was maintained by spraying water on the floor and walls of the room.

Biological efficiency, productivity and carbon/nitrogen relationship

Evaluation of fresh mass in grams, productivity on a percentage wet basis and biological efficiency as a percentage were determined according to Bernardi et al. (2007). The carbon/nitrogen relationship of the substrates was measured according to the Walkey-Black and Semi-micro-Kjeldahl methods (Tedesco et al., 1995).

Bromatological analysis

Mushrooms were characterized, in triplicate samples, in relation to their centesimal composition according to Analytical Standards of the Adolfo Lutz Institute (1985). The carbohydrate determination was obtained by subtracting from 100 the sum of the other parameters.

Statistical analysis

Analysis of variance and Tukey's test was applied to compare means, using the statistical program SANEST (Zonta and Machado, 1984). Significance was tested at the $p < 0.05$ level.

Results and Discussion

Productivity and biological efficiency rates

It was apparent that the rice straw and elephant grass substrates differed significantly on the first and second flows, respectively (Table 1), with the elephant grass standing out from the other treatments. The substrates with the highest productivity were rice straw with the first flow and elephant grass with the second flow, where the elephant grass stood out from the others based on all the flows. The rice straw (first flow) and the elephant grass (second flow) were significantly higher than other treatments based on biological efficiency.

With the second flow, the treatment with elephant grass had the highest averages of fresh mass, productivity and biological efficiency, being 86.40% higher than the lowest averages obtained using sugarcane bagasse. During the production flows, there was a decrease in the values of the variables (Table 1). For example, using the sugarcane bagasse, the decrease reached 100% and there was no fruiting in the second flow due to a contamination on the substrate. Similar results were described by Job (2004), who used coffee spent residues for production of *P. ostreatus*. Bernardi et al. (2007) described a reduction in the fresh mass, productivity and biological efficiency of *P. ostreatus*, *P. ostreatoroseus* and *P. citrinopileatus*, through three production flows. Rossi et al. (2003) described three production flows during the cultivation of shiitake, where there was increased biological efficiency and the average weight of mushrooms produced during the flows, thus demonstrating differences in the cultivation of different species of mushrooms.

Table 1 Fresh mass, productivity and biological efficiency of *Pleurotus sajor-caju* (strain PSC96 / 03) grown on different pasteurized substrates

Variables	Substrate	Flow number		Total
		1°	2°	
Fresh Mass (g)	Elephant grass	178.20^{bA}	54.20^{aB}	232.40^{a}
	Sugarcane bagasse	31.60^{gA}	0.00^{gB}	31.60^{g}
	Rice straw	185.20^{aA}	28.00^{fB}	213.20^{b}
	Elephant grass + Sugarcane bagasse	129.20^{fA}	32.60^{dB}	161.80^{f}
	Elephant grass + Rice straw	139.40^{dA}	39.80^{cB}	179.20^{d}
	Sugarcane bagasse + Rice straw	138.60^{eA}	29.20^{eB}	167.80^{e}
	Elephant grass + Sugarcane bagasse+ Rice straw	160.20^{cA}	46.00^{bB}	206.20^{c}
CV (%)				19.24
Productivity (%)	Elephant grass	17.82^{bA}	5.42^{aB}	23.24^{a}
	Sugarcane bagasse	3.16^{gA}	0.00^{gB}	3.16^{g}
	Rice straw	18.52^{aA}	2.80^{fB}	21.32^{b}
	Elephant grass + Sugarcane bagasse	12.92^{fA}	3.26^{dB}	16.18^{f}
	Elephant grass + Rice straw	13.94^{dA}	3.98^{cB}	17.92^{d}
	Sugarcane bagasse + Rice straw	13.86^{eA}	2.92^{eB}	16.78^{e}
	Elephant grass + Sugarcane bagasse+ Rice straw	16.02^{cA}	4.60^{bB}	20.62^{c}
CV (%)				18.22
Biological efficiency (%)	Elephant grass	71.28^{cA}	21.68^{aB}	92.96^{c}
	Sugarcane bagasse	15.80^{gA}	0.00^{gB}	15.80^{g}
	Rice straw	92.60^{aA}	14.00^{fB}	106.60^{a}
	Elephant grass + Sugarcane bagasse	57.42^{fA}	14.48^{eB}	71.90^{f}
	Elephant grass + Rice straw	61.96^{eA}	17.70^{cB}	79.64^{e}
	Sugarcane bagasse + Rice straw	69.30^{dA}	14.60^{dB}	83.90^{d}
	Elephant grass + Sugarcane bagasse+ Rice straw	74.18^{bA}	21.30^{bB}	95.48^{b}
CV (%)				17.58

CV = coefficient of variation; Flow times = 45 d. Means (n = 5) in the same column superscripted by the same lowercase letter/means in the same row superscripted by the same uppercase letter were not significant different ($p > 0.05$, Tukey's test).

The basidioma coloring ranged from light gray to dark brown as shown in Fig. 1 The basidiomata of *P. sajor-caju* (PSC96/03) were a light gray color when produced on substrates elephant grass, mixed of sugarcane bagasse and rice straw and mixed of elephant grass + sugarcane bagasse + rice straw. However, the basidioma were light brown after cultivation using bagasse sugarcane and a mixture of elephant grass and rice straw. Finally, rice straw and a mixture of elephant grass and sugarcane bagasse substrates produced dark brown mushrooms, especially with the rice straw. The coloring of the basidioma is economically important as consumer preference is for fruiting bodies with darker and stronger coloring (Michael et al., 2011). There is a wide range of substrates used for the cultivation of *Pleurotus* sp. in the literature, but there are few studies related to the possible color differences of produced mushrooms, as studies have been more related to the light intensity. The current verified visual differences in the coloration of *P. sajor-caju* (PSC96/03) mushrooms.

Therefore, depending on the conditions of cultivation (substrate availability, hand labor and the need for larger amounts of mushrooms), it may be more feasible to eliminate the substrates after the first production flow instead of performing the early induction process for a second production stream. Lechner and Albertó (2011) reported a biological efficiency of 80–120% for cultivation of *P. ostreatus* using wheat straw and sawdust supplemented with *Salix* sp., which was similar to the current results, except for the sugarcane bagasse. The low values obtained with the sugarcane bagasse substrate during this experiment can be attributed to the contamination with *Trichoderma* sp. and other substrate contaminants that occurred within the first days of fruiting. The contamination during cultivation in the sugarcane bagasse substrate occurred only when used in its pure form and was not observed when mixed with other substrates. Although contaminated by competing fungi, the mushroom mycelium showed some growth and colonization of the substrate, thereby producing a few basidiomata with regard to fresh mass. This contamination could have been associated with the fact that the sugarcane bagasse substrate had higher concentrations of sugar, which can be easily assimilated and metabolized by competing fungi during mushroom cultivation. Therefore, the competition established between *Trichoderma* sp. and *P. sajor-caju* only decreased the crop yield, considering that *P. sajor-caju* is one of the most competitive species and can adapt to various substrates.

Pasteurization, even when severe, only decreased the amounts of contaminants allowing the best mushroom growth. However, changing the nature of the substrate affected the cultivation yield (Gea, 2001; Royse and Sanchez-Vazquez, 2001; Bernardi et al., 2007). Vieira and Andrade (2016) analyzed *P. ostreatus* cultured in different raw materials and under substrate preparation conditions. They noted that supplementation improved the yield and biological efficiency in all substrates tested, as the biological efficiency was influenced by the conditions and substrate formulation. Sánchez et al. (2002) obtained maximum biological efficiency (78.73%) by cultivating *Pleurotus* spp. in by-products of wine production, which was only higher in the current study results with sugarcane bagasse (15.80%), and elephant grass + sugarcane bagasse (71.90%). However, Siqueira et al. (2011) achieved high biological efficiency of *P. sajor-caju* on two different substrates (banana stalk and Bahia grass) without requiring supplementation. Banik and Nandi (2004) obtained 120% biological efficiency using rice straw added with manure (1:1) to *P. sajor-caju* cultivation, while Moda et al. (2005) evaluated the cultivation of *P. sajor-caju* in pasteurized sugarcane bagasse and reported a biological efficiency of 13.86%, which were lower than those obtained in the current work with the same type of substrate. Those authors observed that a mineral supplementation increased the average biological efficiency in mushroom production (30.0%). Based on the results obtained in the present study and reported in the literature, it appears that different factors (besides the experimental conditions used in each test) can directly influence the biological efficiency during the production process, such as the relationship between the mushroom species, the type of substrate and whether or not there is supplementation.

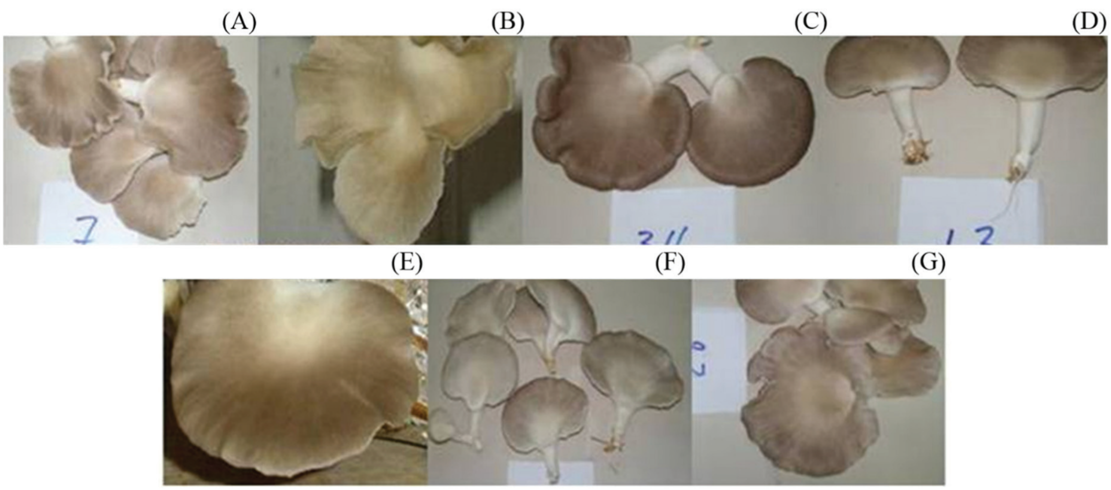

Fig. 1 Basidiomas of *Pleurotus sajor-caju* (PSC96 / 03) produced on different substrates: (A) elephant grass; (B) sugarcane bagasse; (C) rice straw; (D) elephant grass + sugarcane bagasse (1:1); (E) elephant grass + rice straw (1:1); (F) sugarcane bagasse + rice straw (1:1) and (G) elephant grass + sugarcane bagasse + rice straw (1:1:1)

Carbon/nitrogen analyses

Mixed the bagasse with other substrates significantly improved the mushroom yield than when only bagasse was used. The carbon /nitrogen (C/N) values are displayed in Table 2. The highest C/N ratio was achieved using the elephant grass substrate (162:1) which was high for *Pleurotus* sp. compared with Eira and Minhoni (1997), who reported a C/N ratio of 80–100:1 for the cultivation of this kind of mushroom. However, this substrate also produced the highest total fresh mass of mushrooms, as well as the highest productivity among all the substrates. Rice straw, which was the substrate with the lowest C/N ratio (53:1), had the highest biological efficiency. When analyzing productivity and biological efficiency (Table 1) and the initial substrate C/N relationship N (Table 2) of mushrooms produced during this work, it can be seen that the use of elephant grass for *P. sajor-caju* (PSC96/03) cultivation did not require supplementation with other materials to obtain higher levels of nitrogen sources. This was contrary to Sturion (1994), who mentioned that the existence of a lower C/N rate was more favorable in the cultivation substrate in the development stage of the basidioma. Zanetti and Ranal (1997) considered that if on the one hand, the low N content lowers productivity, then on the other, it also adversely affects basidioma production. It is difficult to determine the optimal concentration of N to mycelium to maximize basidioma production because of divergences in the methods and the calculations.

Table 2 Carbon/nitrogen ratios of the substrates used for growing *Pleurotus sajor-caju* (PSC96 / 03)

Substrate	Carbon/nitrogen ratio
Elephant grass	162:1
Sugarcane bagasse	143:1
Rice straw	53:1
Elephant grass+ Sugarcane bagasse	156:1
Elephant grass+ Rice straw	102:1
Sugarcane bagasse+ Rice straw	93:1
Elephant grass+ Sugarcane bagasse+ Rice straw	121:1

Therefore, according to some authors there is a need for supplementation of substrates in the cultivation of mushrooms, in order to increase the values of productivity and biological efficiency. Thus, Vogel and Salmones (2000) added 5.5% soy flour to the dry weight of the wheat straw substrate for the cultivation of *Pleurotus spp.* strains IE-227, 1314 and IE-226, and observed biological efficiency levels of 58.8%, 64.8% and 80.47%, respectively. In another experiment, the same authors used a commercial supplement, instead of soy flour, and found that the biological efficiency of the IE-226 strain increased to 99.3%. However, the resulting C/N ratios, after the addition of nitrogenous compounds, were not mentioned in both these studies. Sunflower seed hulls supplemented with $NH4^{+}$ to produce *P. ostreatus* at concentrations of 0 to 750 ppm, according to Curvetto et al. (2002), increased the productivity of this species by up to 50%, as it promoted mycelial development by adapting the C/N ratio of the used substrate.

One possibility discussed by Royse (2002) is the adequacy of the C/N ratio, which relates the supplementation of substrates with different nutrients as a determining factor for the production of *P. cornucopiae*. Wang et al. (2001) reported increased mushroom biological efficiency with supplementation of barley straw substrate with up to 45% wheat bran for the cultivation of *P. ostreatus*. In cultivation of *P. sajor-caju,* Dias et al. (2003) obtained a biological efficiency of 51% with pure corn straw and 83% with 10% wheat bran supplementation. However, in a study using bean straw, these same authors found there was no difference in the biological efficiency between pure bean straw and bean straw with the supplements described above and concluded that it was not necessary to supplement bean straw for the cultivation of *P. sajor-caju*. Banik and Nandi (2004) described increased biological efficiency of *P. sajor-caju* cultivated on rice straw supplemented with manure (1:1), but as this ratio was increased, the biological efficiency decreased.

The current results showed that *P. sajor-caju* produced more in the first flow with rice straw and in the second with elephant grass. In the sum of both flows, the elephant grass performed better with regard to fresh mass and productivity while the rice straw had higher rates of biological efficiency. Both substrates were viable for mushroom cultivation. Sugarcane bagasse is the poorest substrate for the cultivation of mushroom according to the results using this technique. The sugarcane bagasse is probably most fit for the composition of substrates that are subjected to composting, as it is used in the cultivation of *Agaricus blazei* and even *Pleurotus* spp. in composted substrate (Moda et al., 2005; Valle et al., 2014).

Bromatological parameters

After carrying out bromatological analysis (Table 3), the mushrooms produced in the substrate consisting of elephant grass (50%) and sugarcane bagasse (50%) showed the highest levels of moisture (91.41%) and had higher ether extract rates (5.65%). This greater amount of ether extract is unwanted when aiming for healthy food. Mushrooms produced in the substrate consisting of elephant grass, sugarcane bagasse and rice straw (one-third each), produced a higher amount of dry matter (14.37%) than using any substrate alone. The highest contents of crude protein (42.13%) and ash (7.97%) were obtained from mushrooms produced on the sugarcane bagasse substrate. The highest proportion of carbohydrates (58.66%) and crude fiber (31.33%) were found in elephant grass + rice straw and rice straw substrate, respectively. Bonatti et al. (2004) cultivated *P. ostreatus* and *P. sajor-caju* in banana straw and rice straw and reported that there were lower values of total protein (13.0%) and fiber (9.6%) with *P. sajor-caju* cultivation in rice straw, in comparison to the current study. These difference between the studies may have been related to the conditions the substrates were subjected to (pasteurization or sterilization by autoclaving) and the environmental conditions for fruiting body formation (temperature and humidity).

Table 3 Physico-chemical composition (% dry basis) of *Pleurotus sajor-caju* (PSC 96/03), produced on different pasteurized substrates

Substrate	Humidity	Dry mass	Crude protein	Ash	Ether extract	Carbohydrates	Crude fiber
Elephant grass	90.08^{b}	9.92^{f}	23.45^{g}	6.96^{b}	2.71^{d}	42.27^{b}	24.61^{c}
Sugarcane bagasse	88.63^{e}	11.37^{c}	42.13^{a}	7.97^{a}	1.82^{f}	35.57^{d}	12.51^{f}
Rice straw	89.63^{c}	10.37^{e}	28.01^{c}	6.48^{f}	1.49^{g}	32.69^{g}	31.33^{a}
Elephant grass + Sugarcane bagasse	91.41^{a}	8.59^{g}	26.35^{e}	6.95^{c}	5.65^{a}	34.50^{e}	26.55^{b}
Elephant grass + Rice straw	89.57^{d}	10.43^{d}	23.92^{f}	6.78^{e}	1.95^{e}	58.66^{a}	8.69^{g}
Sugarcane bagasse+ Rice straw	86.92^{f}	13.08^{b}	30.92^{b}	6.95^{c}	3.77^{b}	33.85^{f}	24.51^{d}
Elephant grass + Sugarcane bagasse+ Rice straw	85.63^{g}	14.37^{a}	27.79^{d}	6.91^{d}	2.98^{c}	38.71^{c}	23.91^{e}
CV (%)	10.76	12.74	13.98	11.24	15.32	12.87	16.67

CV = coefficient of variation. eans ($n = 5$) in the same column superscripted with the same lowercase letter were not significantly different ($p > 0.05$, Tukey's test),

Based on the current results, it can be concluded that *P. sajor-caju* (PSC96/03) produced higher amounts of fresh mass and higher productivity and biological efficiency when cultivated with elephant grass substrate. On other hand, sugarcane bagasse in its pure form was rapidly contaminated, diminishing the *P. sajor-caju* (PSC96/03) yield. The mushrooms produced in the elephant grass + sugarcane bagasse substrate had high ether extract values compared to the other treatments. It should be noted that the nature or type of the substrate used in cultivation altered the color of the mushroom.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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