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Original Article

EFFECT OF GROWTH REGULATORS AND TRACE ELEMENTS ON THE VEGETATIVE GROWTH OF *PLEUROTUS SAPIDUS* QUÉL

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ABSTRACT

Objective: The present study was undertaken to investigate the effect of biochemical sources *viz.,* growth regulators and trace elements on the vegetative growth of *Pleurotus sapidus* Quél. It has a great commercial potential being an edible and wood decaying fungus. Mushrooms need carbon and nitrogen for structural and functional purposes in addition to trace elements, growth regulators and vitamins. Therefore, evaluation of their role in influencing the growth of the mushroom is a necessary aspect to be studied.

Methods: Fresh sporocarps of *P. sapidus* were collected from rotten stumps of *Grevillea robusta* A. Cunn. ex R. Br and its pure culture was raised on Potato Dextrose Agar medium. The malt broth liquid medium at 28±1 °C was used as a basal medium for investigating the role of growth regulators (gibberellic acid, indole-3-acetic acid, indole-3-butyric acid and kinetin) and trace elements (manganese, iron, molybdenum, boron and zinc). Different concentrations of growth regulators and salts with trace elements were added to separate medium flask to compare the growth.

Results: The comparative study of various concentrations of growth regulators and trace elements has shown that the cultures supplemented with 5 ppm gibberellic acid and 5 ppm boron, respectively gave maximum mycelial growth of *P. sapidus.*

Conclusion: The vegetative growth of *P. sapidus* can be enhanced by adding gibberellic acid and boron in the basal medium.

Keywords: Growth regulators, *Pleurotus sapidus*, Trace elements, Vegetative growth

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INTRODUCTION

Pleurotus represents one of the four major mushroom genera harboring edible mushroom species being cultivated on a commercial scale for human consumption. It belongs to Class-*Agaricomycetes*, Order-*Agaricales,* and Family-*Pleurotaceae*. It is recognized by as many as 20 species the world over [1]. Because of the excellent culinary and medicinal potential of this genus, some of its edible species including *P. florida* (Mont.) Singer [2, 3], *P. eous* (Berk.) Sacc. [4], *P. ostreatus* (Jacq.) P. Kumm. [5], *P. sajor-caju* (Fr.) Singer, *P. flabellatus* Sacc., *P. pulmonarius* (Fr.) Quél., and *P. cystidiosus* O. K. Mill. [6] have been evaluated by a number of investigators for their antioxidant, antimicrobial and nutritional profile. Unlike other mushrooms, species of *Pleurotus* show much diversity in their adaptation to varying agro-climatic conditions. Due to the flexible nature of the genus and the capability of its species to occupy a wide range of substrate, it has a large number of cultivable species than any other mushroom genus [7]. It's all the species possess the ability to transform wide range of agricultural waste into edible biomass through simple cultivation technology [8]. *Pleurotus sapidus* is a widely distributed species throughout the world. It has pleurotoid basidiocarp which grows on the substrate in caespitose clusters. It is a wood decaying edible species which is being consumed in many parts of the world. The nutritional and nutraceutical profile of this species is quite comparable to other edible species of Pleurotus including *P. sajor-caju*, *P. flabellatus*, *P. pulmonarius* and *P. cystidiosus* [6]. In nature, it colonizes a wide variety of substrates including wooden logs. Presently it was collected from rotten stumps of *Grevillea robusta* A. Cunn. ex R. Br. from Palampur (Himachal Pradesh, India). So far there is no reference about the effect of growth regulators and trace elements on the vegetative growth of *P. sapidus*. The present work is first of its kind which was primarily undertaken to understand the effect of biochemical sources on the vegetative growth of this mushroom.

MATERIALS AND METHODS

The pure culture of the fungus was raised from the fresh fruit bodies on Potato Dextrose Agar (PDA) medium through tissue culture technique and maintained by periodic transfer on PDA medium test tube slants at 27±1 °C for future use. The culture has been deposited in Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology [IMTECH] Chandigarh, India under MTCC number 109481. The composition of liquid media used is after Tuite [9]. For clarifying the medium from impurities standard methodology has been followed [10]. The Malt Broth liquid medium was used as the basal medium for experiments. From the basal medium, impurities were removed by adding 5 g/l of activated charcoal and autoclaving the medium at 15 psi pressure for 15 min using NSW 227 Caltan autoclave. Four growth regulators namely gibberellic acid (GA), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), kinetin (K) and a mixture of all these, each at 5 ppm, 10 ppm, 15 ppm and 20 ppm concentration were prepared to study their effect on mycelial growth. All these chemicals were purchased from HiMedia Laboratories pvt ltd. India. Stock solutions of all growth regulators except gibberellic acid (GA) were prepared in double-distilled water and stored at 5 °C. Gibberellic acid was first dissolved in 10 ml of acetone and then required dilutions were prepared. The medium flasks were inoculated with the freshly homogenized inoculum and then respective growth regulators were added through Axiva syringe 0.2 µm micropore filters. The inoculated flasks were incubated at 28±1 °C for 13 d and the mycelial dry weight was recorded in milligrams using OHAS Adventure ™balance manufactured by Ohaus Corp. Pine Beck, NJ, USA.

Therefore, to study the effect of trace elements on the vegetative growth of the different fungus salts namely Manganese Sulphate ($MnSO_4.7H_2O$), Ferrous Sulphate ($FeSO_4.7H_2O$), Molybdic Acid ($MoO_3.H_2O$), Boric Acid (H_3BO_3) and Zinc Sulphate ($ZnSO_4.7H_2O$) consisting of five trace elements, viz. Manganese (Mn), Iron (Fe), Molybdenum (Mo), Boron (B) and Zinc (Zn) at 1 ppm, 2 ppm and 5 ppm concentration were used which were supplied by Loba Chemie Pvt Ltd. India

Statistical analysis of result

The data observed during the studies was subjected to statistical analysis. The differences exhibited by the treatments in different

experiments were tested for their significance through analysis of variance followed by t-test. The standard deviation (SD) and standard error (SE) was calculated by employing the following formulae:

$$\bar{x} = \frac{\sum x}{n}$$

$$S.D. = \sqrt{\frac{\sum x^2 - (\sum x)^2/n}{n-1}}$$

$$S.E. = \sqrt{\frac{(SD_1)^2}{x} + \frac{(SD_2)^2}{x}}$$

x = variable

Σ = summation of all observations.

n = number of observations

Σx^2= summation of squares of all Observations

$\overline{X}$ = mean of variables

To test the significance of an observed correlation coefficient, following formula for student t-test was applied:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{S.E.}$$

RESULTS

Evaluation of different growth regulators for mycelial growth

At 5 ppm concentration maximum mycelial growth of 4.78 mg/ml was achieved in gibberellic acid followed by 4.69 mg/ml in kinetic, whereas least growth of 0.93 mg/ml was supported by indole-3-acetic acid. At 10 ppm concentration, the maximum mycelial growth of 4.67 mg/ml was obtained in gibberellic acid and 3.57 mg/ml in kinetin. In comparison least mycelial growth of 1.3 mg/ml was supported by the mixture. At 15 ppm concentration, the mycelial growth of 3.69 mg/ml was recorded in kinetin in comparison to 3.46 mg/ml in control, whereas least mycelial growth of 0.53 mg/ml was supported by indole-3-acetic acid. At 20 ppm concentration, the maximum mycelial growth of 3.50 mg/ml was documented in kinetic followed by 3.5 mg/ml mycelial growth in gibberellic acid and minimum mycelial growth of 0.44 mg/ml was achieved in indole-3-acetic acid. In control, only 3.46 mg/ml mycelial dry weight was recorded. The mean dry weight of the mycelium in different growth regulators along with±SD are depicted in the histogram (fig. 1).

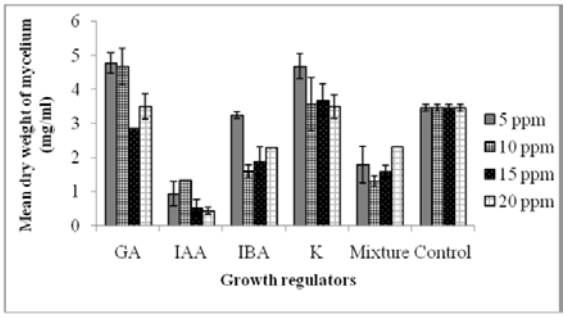

Fig. 1: Histogram showing effect of different concentrations of growth regulators for mycelial growth of *P. sapidus*

At 5 ppm concentration growth achieved in gibberellic acid was significantly higher than growth obtained in indole-3-acetic acid (t=15.27, df=5, p>0.01), indole-3-butyric acid (t=7.968, df=5, p>0.01), mixture (t=8.328,df=5,p>0.01) and control (t=6.875, df=5, p>0.01). In comparison, the growth in kinetin was found to be non-significant. Next best vegetative growth was recorded in kinetin which is significantly higher than growth in indole-3-acetic acid (t=12.659, df=5, p>0.01), indole-3-butyric acid (t=6.428, df=5, p>0.01), mixture (t=7.692, df=5, p>0.01) and control (t=5.491, df=5, p>0.01). In comparison the growth in gibberellic acid was insignificant. Least vegetative growth on dry weight basis was recorded in indole-3-acetic acid which was significantly higher than growth in Indole-3-butyric acid (t=10.593, df=5, p>0.01), kinetin (t=12.659, df=5, p>0.01), mixture (t=2.299, df=5, p>0.01) and control (t=11.552, df=5, p>0.01) (table 1).

Table 1: Matrix table showing t-value at 5 ppm concentration of different growth regulators

S. No.	Growth regulator	Dry wt. (mg/ml)	1	2	3	4	5	6
			GA	IAA	IBA	Kinetin	Mixture	Control
			4.78	0.93	3.25	4.69	1.79	3.46
1	GA	4.78	--	15.27**	7.968**	0.323	8.328**	6.875**
2	IAA	0.93		--	10.593**	12.659**	2.299*	11.552**
3	IBA	3.25			--	6.428**	4.576**	2.1*
4	Kinetin	4.69				--	7.692**	5.491**
5	Mixture	1.79					--	5.235**
6	Control	3.46						--

Values are expressed as mean±standard deviation of three independent experiments. Asterisk (*) indicates significant changes at 0.05 level and double asterisk (**) indicates significant changes at 0.01 level.,

Abbreviations-GA: gibberellic acid, IAA: indole-3-acetic acid, IBA: indole-3-butyric acid.

Maximum vegetative growth at 10 ppm concentration was recorded in gibberellic acid which was significantly higher than growth in indole-3-acetic acid (t=10.918, df=5, p>0.01), indole-3-butyric acid (t=9.109,df=5,p>0.01), mixture (t=10.498, df=5, p>0.01 and control (t=3.858, df=5, p>0.01). In comparison the growth with kinetin was found to be insignificant. Next best vegetative growth was recorded in kinetin which was significantly higher than growth in indole-3-acetic acid (t=4.047, df=5, p>0.05), indole-3-buytric acid (t=3.462, df=5, p>0.01) and mixture (t=4.053, df=5, p>0.01). In comparison the growth with gibberellic acid and control was found to be insignificant (table 2).

As compared at 15 ppm concentration, significantly high growth was recorded in kinetin than in gibberellic acid (t=2.996, df=5, p>0.05), indole-3-acetic acid (t=9.753, df=5, p>0.01), indole-3-butyric acid (t=4.945, df=5, p>0.01), mixture (t=6.976, df=5, p>0.01) and insignificant in control. Next best vegetative growth was recorded in control which was significantly higher than growth in gibberellic acid (t=8.571, df=5, p>0.01), indole-3-acetic acid (t=16.01, df=5, p>0.01), indole-3-butyric acid (t=6.382, df=5, p>0.01) and mixture (t=13.453, df=5, p>0.01). In comparison the growth in kinetin was found to be non significant. Amongst the different growth regulators used, least vegetative growth was recorded in indole-3-acetic acid (table 3).

At 20 ppm concentration maximum mycelial growth was recorded in kinetin which was significantly higher than growth in indole-3-

acetic acid (t=15.00, df=5, p>0.01), indole-3-butyric acid (t=6.173, df=5, p>0.01) and mixture (t=6.020, df=5, p>0.01), whereas mycelial growth achieved in kinetin was found to be insignificant in comparison to growth in gibberellic acid and control. Comparable vegetative growth was recorded in gibberellic acid which was significantly higher than growth in indole-3-acetic acid (t=11.460, df=5, p>0.01), indole-3-butyric acid (t=4.636, df=5, p>0.01) and mixture (t=4.521, df=5, p>0.01). In comparison, the vegetative growth was insignificant in kinetin and control. Least vegetative growth was recorded in indole-3-acetic acid (table 4).

Table 2: Matrix table showing t-values at10 ppm concentration of different growth regulators

S. No.	Growth regulator	Dry wt. (mg/ml)	1	2	3	4	5	6
			GA	IAA	IBA	Kinetin	Mixture	Control
			4.67	1.34	1.6	3.57	1.3	3.46
1	GA	4.67	--	10.918**	9.109**	1.746	10.498**	3.858**
2	IAA	1.34		--	1.843	4.047**	0.412	30.285**
3	IBA	1.60			--	3.462**	1.744	11.772**
4	K	3.57				--	4.053**	0.197
5	Mixture	1.30					--	18.00**
6	Control	3.46						--

Values are expressed as mean±standard deviation of three independent experiments. A double asterisk (**) indicates significant changes at 0.01 level.

Table 3: Matrix table showing t-values at 15 ppm concentration of different growth regulators

S. No.	Growth regulator	Dry wt. (mg/ml)	1	2	3	4	5	6
			GA	IAA	IBA	Kinetin	Mixture	Control
			2.86	0.53	1.89	3.69	1.59	3.46
1	GA	2.86	--	15.533**	4.110**	2.996*	10.583**	8.571**
2	IAA	0.53		--	4.673**	9.753**	5.120**	16.01**
3	IBA	1.89			--	4.945**	1.132	6.382**
4	K	3.69				--	6.976**	0.804
5	Mixture	1.59					--	13.453**
6	Control	3.46						--

Values are expressed as mean±standard deviation of three independent experiments. Asterisk (*) indicates significant changes at 0.05 level and a double asterisk (**) indicates significant changes at 0.01 level.

Table 4: Matrix table showing t-values at 20 ppm concentration of different growth regulators

S. No.	Growth regulator	Dry wt. (mg/ml)	1	2	3	4	5	6
			GA	IAA	IBA	Kinetin	Mixture	Control
			3.5	0.44	2.29	3.50	2.32	3.46
1	GA	3.5	--	11.460**	4.636**	0.000	4.521**	0.148
2	IAA	0.44		--	32.456**	15.00**	32.982**	33.186**
3	IBA	2.29			--	6.173*	0.000	16.710**
4	K	3.50				--	6.020**	0.192
5	Mixture	2.32					--	16.285**
6	Control	3.46						--

Values are expressed as mean±standard deviation of three independent experiments. Asterisk (*) indicates significant changes at 0.05 level and double asterisk (**) indicates significant changes at 0.01 level.

Evaluation of different trace elements for mycelial growth

At 1 ppm concentration, the maximum mycelial growth of 3.63 mg/ml was recorded in control which was followed by 3.16 mg/ml growth in Iron whereas least mycelial growth of 2.25 mg/ml was supported by boron.

In comparison at 2 ppm concentration, the mycelial growth of 3.63 mg/ml was again recorded in control which was maximum followed by 3.62 mg/ml growth in molybdenum whereas least mycelial growth of 2.22 mg/ml was achieved by manganese.

At 5 ppm concentration, the mycelial growth of 3.78 mg/ml was recorded in boron followed by 3.68 mg/ml mycelial growth in the molybdenum while the least mycelial growth of 2.47 mg/ml was supported by iron. Mean dry weight of the mycelium in different trace elements along with±SD are depicted in histogram (fig. 2)

As per t-values calculated at 1 ppm concentration maximum vegetative growth was supported by the control which was significantly higher than growth with molybdenum (t=3.936, df=6, p>0.01), and mixture (t=2.755, df=6, p>0.05). In comparison, the growth in the manganese, iron, boron and zinc was found to be non-significant. Next best vegetative growth was supported by Iron, which was significantly higher than vegetative growth obtained in manganese (t=2.051, df=6, p>0.05) and boron (t=2.146, df=6, p>0.05. In comparison, the growth obtained was insignificant with molybdenum, zinc, mixture and control (table 5).

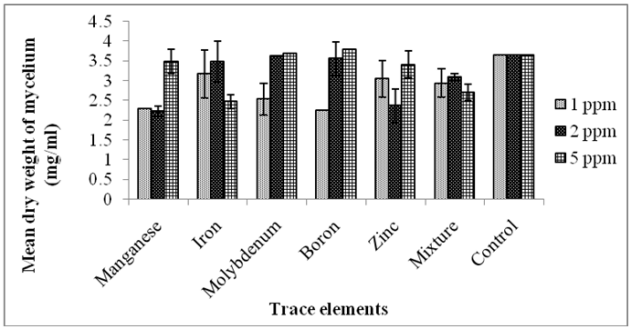

Fig. 2: Histogram showing effect of different concentrations of trace elements on mycelial growth of *P. sapidus*

Maximum vegetative growth at 2 ppm was supported by basal medium (control) which was significantly higher than growth with manganese (t=17.625, df=6, p>0.01), zinc (t=5.120, df=6, p>0.01) and mixture (t=7.857, df=6, p>0.01). In comparison, the growth with iron, molybdenum and boron was found to be nonsignificant. Next best result with respect to vegetative growth was supported by molybdenum, which was significantly higher than growth in manganese (t=17.5, df=6, p>0.01), zinc (t=5.080, df=6, p>0.01) and mixture (t=7.714, df=6, p>0.01). However, the growth with iron, boron and control was found to be nonsignificant (table 6).

At 5 ppm concentration also maximum vegetative growth was obtained in boron which was significantly higher than the growth achieved in iron (t=10.916, df=6, p>0.01) and mixture (t=7.657, df=6, p>0.01). In comparison, the growth with manganese, molybdenum, zinc and control was found to be non-significant. At this concentration, the next best vegetative growth obtained was supported by molybdenum which was significantly higher than vegetative growth obtained in iron (t=10.003, df=6, p>0.01), and mixture (t=6.950, df=6, p>0.01). However, the growth with manganese, boron, zinc and control was found to be non-significant (table 7).

Table 5: Matrix table showing t-values 1 ppm concentration of different trace elements on mycelial growth of *P. sapidus*

S. No.	Trace element	Dry wt. (mg/ml)	1	2	3	4	5	6	7
			Mn	Fe	Mo	B	Zn	Mixture	Control
			2.29	3.16	2.52	2.25	3.04	2.93	3.63
1	Manganese	2.29	--	2.051*	0.815	0.000	2.259*	2.519*	0.000
2	Iron	3.16		--	1.257	2.146*	0.223	0.465	1.108
3	Molybdenum	2.52			--	0.957	1.192	1.078	3.936**
4	Boron	2.25				--	2.379*	2.677*	0.000
5	Zinc	3.04					--	0.263	1.777
6	Mixture	2.93						--	2.755*
7	Control	3.63							--

Values are expressed as mean±standard deviation of three independent experiments. Asterisk (*) indicates significant changes at 0.05 level and a double asterisk (**) indicates significant changes at 0.01 level.

Table 6: Matrix table showing t-values at 2 ppm concentration of different trace elements on mycelial growth of *P. sapidus*

S. No.	Trace element	Dry wt. (mg/ml)	1	2	3	4	5	6	7
			Mn	Fe	Mo	B	Zn	Mixture	Control
			2.22	3.48	3.62	3.55	2.36	3.08	3.63
1	Manganese	2.22	--	3.351**	17.5**	5.115**	0.538	8.037**	17.625**
2	Iron	3.48		--	0.517	0.518	2.528*	1.069	0.408
3	Molybdenum	3.62			--	0.282	5.080**	7.714**	0.000
4	Boron	3.55				--	3.400**	1.821	0.322
5	Zinc	2.36					--	2.790*	5.120**
6	Mixture	3.08						--	7.857**
7	Control	3.63							--

Values are expressed as mean±standard deviation of independent experiments. Asterisk (*) indicates significant changes at 0.05 level and a double asterisk (**) indicates significant changes at 0.01 level.

Table 7: Matrix table showing t-values at 5 ppm concentration of different trace elements on mycelial growth of *P. sapidus*

S. No.	Trace element	Dry wt. (mg/ml)	1	2	3	4	5	6	7
			Mn	Fe	Mo	B	Zn	Mixture	Control
			3.48	2.47	3.68	3.78	3.4	2.7	3.63
1	Manganese	3.48	--	4.056**	0.913	1.369	0.246	3.000*	0.684
2	Iron	2.47		--	10.083**	10.916**	3.470**	1.243	9.666**
3	Molybdenum	3.68			--	0.000	1.166	6.950**	0.000
4	Boron	3.78				--	1.583	7.657**	0.000
5	Zinc	3.4					--	2.517*	0.958
6	Mixture	2.7						--	6.559**
7	Control	3.63							--

Values are expressed as mean±standard deviation of three independent experiments. Asterisk (*) indicates significant changes at 0.05 level and double asterisk (**) indicates significant changes at 0.01 level.

DISCUSSION

During the present investigation, gibberellic acid at 5 ppm concentration has shown maximum mycelial growth of 4.78 mg/ml for *P. sapidus.* In comparison minimum, mycelial growth of 0.44 mg/ml was supported by indole-3-acetic acid at 10 ppm [11]. There is a report of increased mycelial dry weight in the case of *P. eous* with 0.5 ppm GA [7]. So far there is no work of this type by any worker on *P. sapidus*. Out of all the concentrations of each trace element and the mixture used, maximum mycelial growth (3.68 mg/ml) was obtained at 5 ppm concentration of boron and least growth (2.22 mg/ml) was obtained in manganese at 2 ppm concentration. Hence out of all the trace elements evaluated boron at 5 ppm concentration was found to be the most suitable trace element for promoting the vegetative growth of *P. sapidus.* Eswaran and Ramabadran obtained maximum mycelial dry weight with the addition of copper sulfate (3 ppm) in glucose-asparagine solution in case of *P. eous* [11]. Eswaran and Ramanujam applied gibberellic acid (GA), indole-3-acetic acid (IAA) and 6-benzyl aminopurine (BAP) at 1 ppm, 10 ppm, 100 ppm and 200 ppm concentration to *Pleurotus* sp. cultivated on beds and the results indicated increased number and weight of the sporophores including increased

biological efficiency [12]. Significant increase in number, the weight of sporophores and biological efficiency has been reported when GA at l00 ppm concentration was applied. Tandon and Sharma reported maximum mycelial growth in case of *Calocybe indica* Purkayastha and A. Chandra in medium with 10 ppm of gibberellic acid [13]. Increased mycelial growth in 20-40 ppm of gibberellic acid has been documented in case of *Lentinus edodes* (Berk.) Singer [14]. Best mycelial growth on dry weight basis has been reported in *L. connatus* Berk. at 5 ppm concentration of indole-3-butric acid in the basal medium [15]. With the addition of 2 ppm manganese in the form of manganese sulphate as trace element and 50 ppm gibberellic acid as a growth regulator in the basal medium, maximum vegetative growth has been achieved in the case of *Flammulina velutipes* (Curtis) Singer [16]. Calcium, magnesium and potassium are reported to stimulate the good growth of *P. florida* while iron and zinc in a very low concentration are documented to be required for its propagation [17]. These elements are reported for use as a supplement in the growth media to produce high mycelial yield needed for spawning and fruit body production of this edible mushroom [17]. In the case of *Lentinus cladopus* Lév. the enhancement of vegetative growth was documented in 10 ppm concentration of indole-3-butyric acid (IBA) and 15 ppm concentration of iron [18]. In the case of *L. squarrosulus* Mont. at 20 ppm concentration maximum mycelial growth of 17.55 mg/ml was recorded in gibberellic acid (GA) while least mycelial growth was documented in indole-3-butyric acid (IBA) [19]. Some of these observations confirm the conclusions of the present investigation supporting increased vegetative growth when the basal medium is supplemented with 5 ppm concentration each of gibberellic acid and boron.

CONCLUSION

The results obtained in the present study revealed that gibberellic acid at 5 ppm and boron at 5 ppm concentration supported the maximum vegetative growth of *P. sapidus*. The present findings have helped us to understand the biochemical requirements of *P. sapidus* for enhancing the vegetative growth of this mushroom in the basal medium.

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CONFLICT OF INTERESTS

Declared none

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