Pertanika J. Trop. Agric. Sci. **19(2/3)**: 171-174 (1996)
ISSN: 0126-6128

Mycelial Growth and Germanium Uptake by Four Species of *Ganoderma*

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Keywords: ***Ganoderma tsugae, G. subamboinense* var. *laevisporium, G. tropicum, G. lucidum*, germanium**

ABSTRAK

Miselia dari empat spesis Ganoderma *(*G. tsugae, G. subamboinense *var.* laevisporium *ATCC 52419*, G. tropicum *dan* G. lucidum*) telah ditumbuhkan dalam medium cecair yang mempunyai kepekatan germanium (Ge) yang berlainan selama 20 hari pada suhu 28°C. Didapati bahawa kadar pertumbuhan dan berat kering miselia keempat-empat spesis semakin menurun dengan penambahan kepekatan Ge dalam medium walaupun peratusan pengambilan Ge oleh miselia semakin bertambah. Tahap pertahanan miselia terhadap Ge di dalam medium pertumbuhan berbeza untuk keempat-empat spesis* Ganoderma. *Untuk setiap spesis, kepekatan optima Ge di dalam medium telah ditentukan sebagai 100mg/l untuk mendapatkan keadaan optima bagi pengambilan Ge serta pertumbuhan miselia yang optimum.*

ABSTRACT

Four Ganoderma *species (*G. tsugae, G. subamboinense *var.* laevisporium, *ATCC 52419*, G. tropicum *and* G. lucidum*) were incubated in liquid medium containing different concentrations of germanium (Ge) for up to 20 days at 28°C. Increasing the Ge concentration of the medium resulted in a gradual decrease in the growth of the fungal mycelium. However, the Ge content in the mycelium increased with increasing Ge concentration. Different species recorded different levels of tolerance towards the Ge. In each case, the optimum concentration of the incorporated Ge in the medium was established as 100 mg/l for both optimal uptake of Ge by the fungal mycelium and optimal mycelial growth.*

INTRODUCTION

Traditionally in the Orient, *Ganoderma* has been considered an "elixir of life". Today, there is a considerable body of contemporary research that shows that certain species of *Ganoderma* are highly effective medicinal agents as a popular remedy to treat hepatopathy, chronic hepatitis, nephritis, hypertension, hyperlipedemia, arthritis, neurasthenia, insomnia, bronchitis, asthma, gastric ulcer, arteriosclerosis. leukopenia, diabetes, anorexia, mushroom poisoning and debility due to prolonged illness (Willard 1990). The medicinal value of *G. lucidum* is closely linked to the presence of the following compounds: organic-Ge, polysaccharides, triterpenoids and adenosine (Tong 1995).

In Malaysia, studies on cultivation techniques of a suitable strain of *G. lucidum* well adapted to the local climatic conditions (Tong and Chen 1990), its growth characteristics and Ge uptake by the mycelium (Tong *et al.* 1994a) as well as the fruiting bodies of this fungus (Tong *et al.* 1994b) have been carried out. Because of the paucity of information on the uptake of Ge by other species of *Ganoderma* of com-

mercial value, the present study was undertaken to ascertain differences, if any, in their uptake of Ge.

MATERIALS AND METHODS

Cultures

Four species, *G. tsugae, G. subamboinense* var. *laevisporium* (ATCC 52419), *G. tropicum* and *G. lucidum* were obtained from Taiwan and maintained on potato dextrose agar (PDA) at 28°C and subcultured every 3 weeks.

Uptake of Ge by Fungal Mycelium Grown in Liquid Medium

Samples (100 ml) of potato dextrose broth containing each of the following concentrations (50, 100, 200, 300, 400 mg/l) of GeO_2 were poured into 1-l flat bottles and sterilized at 121°C, 15 psi for 15 min. Each bottle was then inoculated with three agar mycelium discs (1.3 cm diam) of a ten-day-old culture and incubated in the dark at 28°C for 20 days. The mycelium was later harvested through pre-weighed Whatman No. 1 filter paper and washed with several changes of double distilled deionised water (200 ml). The filter paper, together with the washed mycelium, was dried at 60°C for 48 h. The dry weight of the mycelium was recorded.

Analysis of Germanium

Fungal mycelium samples were ashed at 700-800°C for 1 – 3 hours. The ash was then dissolved in 5M HCl. Germanium in the solution was determined by hydride generation – inductively coupled plasma atomic emission spectrometry (ICP-AES) using the Labtest Plasmascan 710 instrument. The method of analysis was adapted from the method for arsenic analysis (Lee and Low 1987).

A spectropure (Aldrich Chemical Co. Inc.) Ge atomic absorption standard solution of 990 mg/l was used as the stock solution. The standard solutions used in the analysis were prepared by sequential dilution from the stock solution. All reagents used were analytical grade. A solution of 2% sodium borohydride in 0.1% sodium hydroxide was prepared daily from $NaBH_4$ pellets.

The rate of uptake (%) of Ge is calculated as:

$$\frac{\text{Amount of Ge in known dry weight of mycelium}}{\text{Amount of Ge in 100 ml medium}} \times 100$$

RESULTS AND DISCUSSION

Effect of Ge on Mycelial Growth in Liquid Medium

With increasing amounts of GeO_2 incorporated into the medium, there was a corresponding decrease in the dry weight of the mycelium (*Fig 1*). The effect was most obvious for *G. tsugae*, which experienced a continuous sharp drop in the mycelial dry weight at concentrations above 50 mg/l of GeO_2. Similarly for *G. lucidum*, the growth of the mycelium was

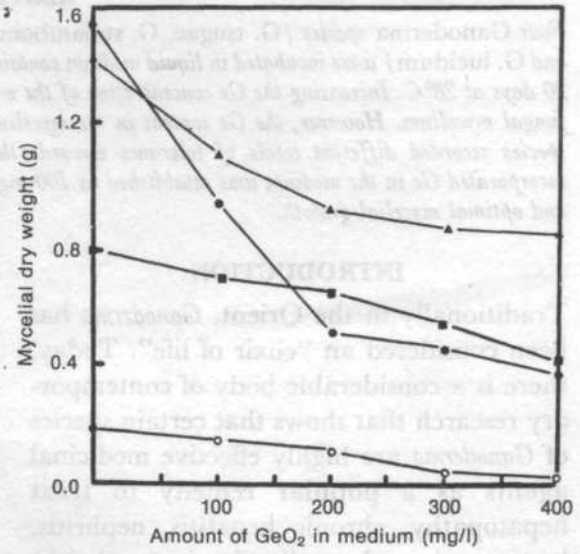

Fig 1. Mycelial growth of Ganoderma *species in liquid medium containing different concentrations of GeO_2 incubated for 20 days at 28°C. Results represent average of triplicates.*

● G. tsugae ▲ G. tropicum ○ G. lucidum
■ G. subamboinense *var.* laevisporium

TABLE 1
Effect of GeO_2 in liquid medium on the uptake of Ge by mycelium after 20 days of incubation at 28°C

Species	Conc. of GeO_2 in 100 ml liquid medium (mg/l)	Mycelial dry weight (g)	Amount of Ge in mycelium (mg/kg)	Uptake of Ge (%)
G. tsugae	50	0.79	752 + 5	11.8
	100	0.70	1892 + 15	13.3
	200	0.68	2456 + 22	8.3
	300	0.54	3855 + 127	6.9
	400	0.38	4312 + 90	4.0
G. subamboinense	50	1.43	34 + 4	1.0
var *laevisporium*	100	1.10	99 + 3	1.1
	200	0.93	175 + 11	0.8
	300	0.87	260 + 9	0.7
	400	0.82	310 + 8	0.6
G. tropicum	50	1.53	235 + 7	7.2
	100	0.94	1080 + 22	10.1
	200	0.52	2072 + 15	5.4
	300	0.46	2715 + 43	4.2
	400	0.37	4002 + 90	4.3
G. lucidum	50	0.12	687 + 18	6.4
	100	0.11	1796 + 14	8.0
	200	0.10	2845 + 31	6.0
	300	0.05	4251 + 112	3.2
	400	0.04	5431 + 87	2.6

Results represent average of triplicates

retarded considerably. This may be due to toxicity at high concentrations. Only extremely sparse growth occurred at concentrations of 250 mg/l of GeO_2 and at concentrations above 300 mg/l of GeO_2 there was no growth of the mycelium at all until the tenth day of incubation (visual observation). The limited growth which commenced after this lag period may probably be due to the induction of some detoxification mechanism.

The growth of the mycelium for *G. subamboinense* var. *laevisporum* was least affected by the amount of GeO_2 in the medium and there was a more gradual decline in the mycelial dry weight than in the other species (*Fig 1*). Of the four species tested, *G. tropicum* produced the most abundant mycelial growth at Day 20 and the effect of GeO_2 was more gradual above 200 mg/l of GeO_2 but significantly decreased between 50 and 200 mg/l.

Uptake of Ge by Fungal Mycelium in Liquid Medium

Table 1 shows that in general, Ge uptake by the mycelium increased with increasing amounts of GeO_2 in the growth medium. Of the four species tested, *G. subamboinense* var. *laevisporium* assimilated least Ge into the mycelium, which may explain why its mycelial growth is least affected by increasing quantities of GeO_2 in the medium. At 400 mg/l of the GeO_2 in the medium, *G.*

tsugae and *G. tropicum* took up almost 13 times, while *G. lucidum* took up 17 times more Ge than *G. subamboinense* var. *laevisporium*. However, it was noted that the rate of uptake (%) of Ge by the mycelium increased to a maximum at 100 mg/l of GeO_2 in all species and then decreased with increasing concentration of GeO_2 in the medium. The highest rate of uptake recorded was 13.3, 10.1, 8.0 and 1.1% for *G. tsugae*, *G. tropicum*, *G. lucidum* and *G. subamboinense* var. *laevisporium*, respectively.

Thus, under the conditions studied, it was deduced that for maximum production of fungal mycelium with optimal Ge content, the optimum concentration of GeO_2 to be added to the medium was 100 mg/l.

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(Received 2 May 1996)

(Accepted 20 August 1996)