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The optimal Factors for the Mycelial Growth of *Sparassis crispa*

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꽃송이 버섯의 균사생장을 위한 최적요인

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ABSTRACT: This study was carried out to obtain the basic data for artificial culture of *Sparassis crispa*. The optimal conditions for the mycelial growth were 25°C and pH 4, respectively. The mycelial density of *S. crispa* was most compact in the Hamada media, whereas colony diameter was prominent in the Hoppkins media. Carbon sources such as maltose, arabinose and mannitol were favorable for stimulating a mycelial growth of *S. crispa*. Glycine, one of nitrogen sources also appeared to be favorable to the mycelial growth. The optimum C/N ratio was about 20 : 1 in case that 1% glucose as carbon source was mixed with the basal medium. Fumaric acid or lactic acid as organic acid was most favorable to the mycelial growth.

KEYWORDS: ***Sparassis crispa*, Nutritional sources, Mycelial growth**

Sparassis crispa (formerly *S. ramosa*), one of edible fungi or butt-rot fungi belongs to *Corticiaceae* of *Basidiomycetes* (Igarashi and Takeuchi, 1985; Kim *et al.*, 1991; Mao and Jiang, 1993). *S. crispa* has been reported to cause a brown root-and butt-rot in living cornifers, closely similar to damage caused by *Phaeolus schweinitzii* (Igarashi and Takeuchi, 1985; Woodward *et al.*, 1993). Both fungi can cause significant losses in managed cornifer forest, but without basidiocarps or cultures, it is difficult to determine the fact which species is responsible for the damage (Phillips and Burdekin, 1982). However, *S. crispa* can produce an antifungal metabolite such as sparassol (methyl-2-hydroxy-4 methoxy-6-methylbenzoate) which is antagonistic to *Cladosporium cucumerinum* causing foliar diseases (Wedekind and Fleischer, 1923; Woodward *et al.*, 1993). Especially, the fruiting bodies of *S. crispa* have been reported to exhibit an outstanding effect for curing human diseases such as a gastric ulcer and esophageal cancer (Mao and Jiang, 1993).

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Since 1923, various researches of *S. crispa* have been intensively carried out to control human diseases or foliar diseases (Wedekind and Fleischer, 1923; Igarashi and Takeuchi, 1985; Kim *et al.*, 1991; Woodward *et al.*, 1993). However, one of urgent problems may be focused on the attempt of its mass production which can support previous researches or satisfy various demands of many users as edible fungi.

Therefore, this study was carried out to find the possibility for an artificial cultivation of *S. crispa* and obtain basic data for its mass production. This is the first report referring to cultural characteristics of *S. crispa*.

Materials and Methods

Cultures

The strain of *S. crispa* has been maintained at the laboratory of microbiology in the Department of Applied Biology, Dongguk University. To prepare a lot of samples which could facilitate various tests, *S. crispa* was transferred to PDA agar plate and was incubated at 25°C until it exhibited a full growth in the dark condition. Unless otherwise stated, all the tests which the strain was used were performed at least twice (Chang *et al.*, 1995; Chi *et al.*, 1996).

Screening of favorable culture media

Fourteen different culture media were used to investigate a favorable growth of *S. crispa*, and adjusted to pH 6.0 before a high-pressure sterilization (Table 1). After 14 different culture media were autoclaved for 15 minutes at 121°C (15 psi pressure), 20 m*l* of each agar solution was aseptically poured into a petri-dish. A 5 mm diameter plug of an inoculum was removed with cork borer from 15 days old cultures of *S. crispa* grown on PDA medium (potato 200g, dextrose 20g and agar 20g/distilled water 1L), placed in the center of each agar plate and incubated for 15 days at 25°C (Chi *et al.*, 1996).

After 15 days of incubation, the mycelial growth and density were observed.

Screening of favorable nutrient sources

Carbon sources To screen favorable carbon source capable of stimulating mycelial growth of *S. crispa*, the basal medium used was composed of $MgSO_4 \cdot 7H_2O$ 0.05g, KH_2PO_4 0.46g, K_2HPO_4 1.0g, thiamine-HCl 120 μg, agar 20g, and distilled water 1000 m*l* (Sung *et al.*, 1993). Based on each molecular weight of 19 different carbon sources including glucose, each carbon source was added to the basal medium at the concentration of 0.1 M per 1L and mixed throughly (Chi *et al.*, 1996). The basal medium was adjusted to pH 6.0 before high-pressure sterilization, and autoclaved for 15 minutes at 121°C (15 psi pressure). With the basal medium containing each carbon source, all the other processes including the inoculation, incubation and measurement of mycelial density of *S. crispa* were performed according to the method described by Chi *et al.* (1996).

Nitrogen sources Except for the addition of 2% glucose as carbon source per 1000 m*l* of the medium, the basal medium used was made of the same additives as those described by Sung *et al.* (1993). Based on each molecular weight of 17 different nitrogen sources including alanine, each nitrogen source was added to the basal medium at the concentration of 0.02 M (Park, *et al.*, 1995; Chi *et al.*, 1996). The basal medium was adjusted to pH 6.0 before high-pressure sterilization, and autoclaved for 15 minutes at 121°C (15 psi pressure). With the basal medium containing each nitorgen source, all the other processes including the inoculation, incubation and measurement of mycelial density of *S. crispa* were performed according to the method described by Chi *et al.* (1996).

C/N ratio The basal media which D-glucose as carbon source was mixed at the rate of 1, 2, 3 and 4%(w/v) were continually added with $NaNO_3$ as nitrogen source. Finally, the ratios of $NaNO_3$ versus D-glucose in each basal medium were adjusted to the C/N ratio of 10:1, 20:1, 30:1 and 40:1, respectively. The basal media were adjusted to pH 6.0, autoclaved for 15 minutes at 121°C (15 psi pressure), and poured into a peri-dish. Also, all the other processes including the inoculation, incubation and measurement of mycelial density of *S. cispa* were performed according to the method described by Chi *et al.* (1996).

Organic acids After the addition of 2% D-glucose as carbon source and 0.25% arginine as nitrogen source per 1000 m*l* of the medium, each organic acid was mixed in the basal medium. Each of 9 different organic acids used was added at the rate of 0.1%(w/v) per 1000 m*l* of basal medium (Kim *et al.*, 1994; Kang *et al.*, 1994; Chi *et al.*, 1996). The basal media were adjusted to pH 6.0, and autoclaved for 15 minutes at 121°C (15 psi pressure). All the other processes were performed according to the method described by Chi *et al.* (1996).

Measurement of pH PDA medium was used to screen pH value suitable for a favorable growth of *S. crispa*. A 5 mm diameter plug of an inoculum was removed with cork borer from 15 days old cultures of *S. crispa* grown on PDA medium, placed in the center of each agar plate of sterile PDA medium adjusted to the range of pH 4~9 with 1N NaOH or Hcl, and incubated for 15 days at 25°C (Chi *et al.*, 1996). The measurement of mycelial growth was performed according to the method described by Chi *et al.* (1996).

The temperatures To screen the optimal temperature suitable for stimulating a favorable growth of *S. crispa*, potato dextrose broth medium was used. A 5 mm diameter plug of an inoculum was inoculated into 50 m*l* of sterile PD broth medium and incubated for 15 days at 15°C, 20°C, 25°C and 30°C, respectively. The inocula submerged were filtrated through filter paper (Whatman No 2, dia., 9 cm), dried for 24 hours at 80°C, and weighed in the balance (Chi *et al.*, 1996).

Results and Discussions

Cultural conditions of *S. crispa*

Effect of pH To screen pH value suitable for a favorable growth of *S. crispa*, the pH values in the PDA medium were adjusted to intervals of pH 1.0 in the range of pH 4~9. The mycelial growth of *S. crispa* was most favorable at pH 4, whereas there was no a mycelial growth at pH 8 and pH 9 (Fig. 1). Shoji (1996) suggested that pH range suitable for a favorable growth of Genera *Grifola* was from pH 4.4 to pH 4.9. Lee (1986) reported that pH value suitable for a favorable growth of *G. umbellata* could be obtained in the range of pH 4.2~5.8. Also, pH range suitable for *S. crispa* appears to be lower than pH 5.0. The mycelial growth of *S. crispa* appeared to be suppressed in proportion to the rise of pH.

Effect of culture temperatures Based on *S. crispa* which was cultured for 15 days at 4 different temperatures, the mycelial growth was

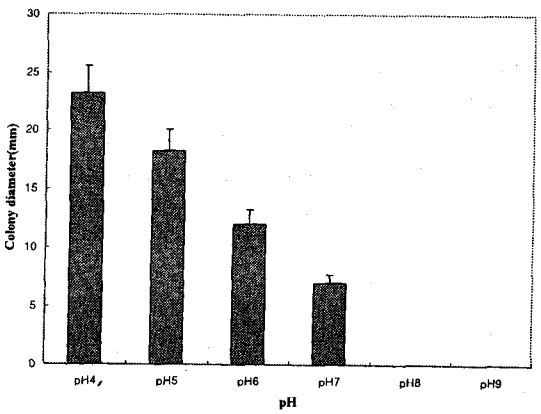

Fig. 1. Mycelial growth of *Sparassis crispa* in the potato dextrose agar at different pHs for 15 days at 25°C.

most favorable in the temperature of 25°C. Woodward *et al.* (1993) clarified that *S. crispa* could be favorably incubated in darkness for 14 days at 22°C. Strickly speaking, the mycelial growth of *S. crispa* was most favorable at 25°C. When the temperature was raised above 25°C, the mycelial growth of *S. crispa* appeared to be suppressed gradually (Fig. 2).

Screening of favorable culture medium Fourteen different culture media were used to investigate a favorable growth of *S. crispa* (Table 1). The mycelial density of *S. crispa* was most compact on the Hamada media,

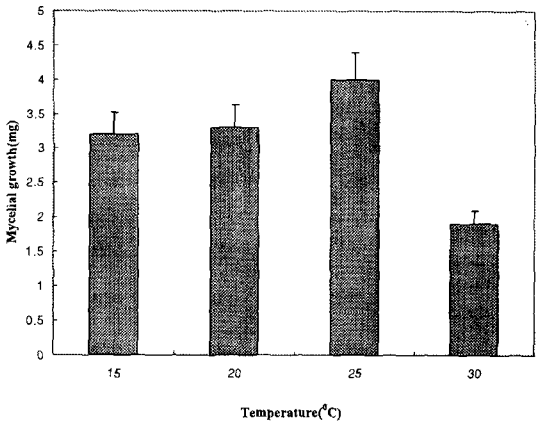

Fig. 2. Mycelia growth of *Sparasis crispa* in the PD broth for 15 days at differen temperatures

Table 1. Compositions of the media used in this experiment

Nutritional reagents	Media (g/l)												
	Hamada	Czapekdox	YM	Henner-berg	Lilly	Modified Lutz	Hopp-kins	PDA	Glucose Peptone	Glucose tryptone	Mushroom complete	Ebi-ose	PD (M)	MEA
Dextrose	20		10					20					20	
Ebiose	5											5		
Yeast ext.	3		3						10	3	2			
Hyponex	3													
Sucrose		30												
Peptone			5						10		2			0.8
Malt ext.			3			10			15				5	
Glucose				50			10		10	5	20			
Maltose					10									13
Asparagine					2									
Potatoes								200					200	
Tryptone										10				
$NaNO_3$		3		2										
K_2HPO_4		1				1					1			
$MgSO_4$		0.5		0.5	0.5	0.1	0.5				0.5			
Kcl		0.5												
$FeSO_4$		0.01												
$CaCl_2$				0.1										
KH_2PO_4				1	1		0.1				0.5			
KNO_3				2			2							
NH_4NO_3						1								
$(NH_4)_2HPO_4$						1								
$MnSO_4$						0.025								
Glycerol														3
Dextrin														3

whereas colony diameter was prominent on the Hopkins media (Table 2). To investigate interations of *S. crispa* with other wood-inhabitating fungi, Woodward *et al.* (1993) used malt extract agar (MEA) as a selective medium which could favorably stimulate a mycelial growth of *S. crispa*. However,it was observed that *S. crispa* appeared to exhibit a poor growth on the Malt extract agar (Table 2). The mycelial growth of 14 different culture media was observed in the range of 4.6~28.2 mm.

Effect of carbon sources Among 19 carbon sources, 16 carbon sources were favorable to the mycelial growth of *S. crispa* as compared with the control (Table 3). The mycelial growth of *S. crispa* was most favorable on the culture media which were supplemented with maltose, one of disaccharides, and recorded colony diameter of 27.2 mm (Table 3). Hong *et al.* (1986) clarified that cellobiose, one of disaccharides was most outstanding for stimulating a mycelial growth of *Ganoderma lucidum*. However, Chang *et al.* (1995) reported that cellobiose was most unfavorable for *Fomitella fraxinea*. Though *G. lucidum* exhibited a favorable growth on the culture media containing cellobiose, the mycelial growth of *S. crispa* was poor and dissimilar to that of *G. lucidum*.

Effect of nitrogen sources It was observed that glycine, one of organic nitrogens appeared to stimulate a mycelial growth of *S.*

Table 2. Mycelial growth of *Sparassis crispa* in different culture media

Culture media	Colony diameter[b] (mm)	
Hamada	21.0 b[a]	C[c]
Czapek	9.4 e-g	ST
YM	11.6 de	C
Hennerberg	15.0 cd	T
Lilly	11.2 d-f	C
Modified Lutz	8.6 e-f	ST
Hoppkins	28.2 a	T
PDA	18.8 bc	C
Glucose peptone	6.0 fg	T
Glucose tryptone	4.6 g	T
Mushroom complete	12.4 de	ST
Ebiose	6.0 fg	T
PD(M)	19.0 bc	C
MEA	5.0 g	T

[a] The different letters are significantly different at p=0.05 according to Duncan's new multiple range test.
[b] The colony diameter was measured after 15 days of incubation.
[c] Mycelial density: C, Compact; S, Somewhat compact; ST, Somewhat thin; T, Thin.

Table 3. Effect of carbon sources for the mycelial growth of *Sparassis crispa* in the basal medium[c]

Carbon source[e]	Colony diameter[d] (mm)	
Glucose	9.6 d-i[a]	T[b]
Galactose	7.6 g-c	T
Fructose	17.4 b	T
Xylose	13.0 cd	T
Arabinose	20.4 b	T
Mannose	8.6 g-i	T
Sorbitol	16.0 c	T
Rhamnose	8.0 g-i	T
Ribose	12.2 c-f	T
Sucrose	12.8 c-e	T
Mannitol	22.6 b	T
Cellobiose	10.8 d-h	T
Lactose	8.8 e-i	T
Raffinose	8.2 f-i	T
Dextrin	11.4 d-g	T
Salicin	6.0 i	T
Adonitol	7.0 hi	T
Trehalose	7.0 hi	T
Maltose	27.2 a	ST
Control	7.2 g-i	T

[a] The different letters are significantly different at p=0.01 according to Duncan's new multiple range test.
[b] Mycelial density: ST, Somewhat thin; T, Thin.
[c] This was made of $MgSO_4 \cdot 7H_2O$ 0.05g, KH_2PO_4 0.46g, K_2HPO_4 1.0g, thiamine-HCl 120 μg, agar 20g, and distilled water 1000 m*l*.
[d] The colony diameter was measured after 15 days of incubation.
[e] Each carbon source was added in the basal medium at the concentration of 0.1 M.

crispa on the culture media (Table 4). There was no mycelial growth on the culture media which were supplemented with nitrogen sources such as methionine, glutamine, urea, histidine, ammonium acetate, calcium nitate, sodium nitrate and potassium nitrate. Generally, it was confirmed that most of 17 different nitrogen sources couldn't exercise a distinct influence upon favorable growth of *S. crispa* (Table 4). Kim *et al.* (1994) clarified that the mycelial growth of *Lentinus lepideus* was more favorable on the culture media containing organic nitrogen than inorganic nitrogen. As described on Table 4, it was not difficult to testify the fact that the mycelial growth of *S. crispa* was more favorable on the culture media containing organic nitrogen than inorganic nitrogen.

Table 4. Effect of nitrogen sources for mycelial growth of *Sparassis crispa* in the basal medium[c]

Nitrogen source[e]	Colony diameter[d] (mm)		
Asparagine	14.8	d[a]	ST[b]
Glutamic acid	18.2	bc	T
Alanine	15.8	cd	ST
Phenylalanine	10.4	e	SC
Valine	16.8	b-d	T
Methionine	0	f	–
Alginine	14.8	d	T
Glycine	21.8	a	SC
Glutamine	19.0	b	T
Urea	0	f	–
Histidine	0	f	–
Ammonium acetate	0	f	–
Ammonium oxalate	9.2	e	T
Ammonium phosphate	17.8	bc	T
Calcium nitrate	0	f	–
Potasium nitrate	0	f	–
Sodium nitrate	0	f	–
Control	21.4	a	T

[a] The different letters are significantly different at p=0.01 according to Duncan's new multiple range test.
[b] Mycelial density: SC, Somewhat compact; ST, Somewhat thin; T, Thin
[c] This was made of $MgSO_4 \cdot 7H_2O$ 0.05g, KH_2PO_4 0.46g, K_2HPO_4 1.0g, thiamine-HCl 120 μg, agar 20g, and distilled water 1000 m*l*.
[d] The colony diameter was measured after 15 days of incubation.
[e] Each nirogen source was added to the basal medium at the concentration of 0.02 M.

Optimum C/N ratio Optimum C/N ratio suitable for a favorable growth of *S. crispa* was observed on the culture media which were adjusted to the ratio of twenty (carbon) to one (nitrogen). On the culture media which were mixed with 1% glucose as carbon source and then adjusted to the C/N ratio of 20 : 1, the most favorable growth of *S. crispa* was not more than colony diameter of 18.6 mm (Table 5). Generally, a gradual rise of carbon concentration such as 1%, 2%, 3% and 4% glucose seemed to suppress a favorable growth of *G. umbellata* on the culture media. Song and Cho (1986) suggested that optimum C/N ratio suitable for a favorable growth of *Lentinula edodes* was observed in the ratio of 30 : 1. However, it was considered that our result was not similar to that of Song and Cho (Table 5).

Effect of organic acids The most favorable growth of *S. crispa* was obtained on the culture media which were supplemented with fumaric acid or lactic acid, and recorded colony diameter of 15.8 mm (Table 6). However, there was no a mycelial growth on the cul-

Table 5. Mycelial growth of *Sparassis crispa* at different C/N ratios in the basal medium[a]

C/N[c] ratio	D-Glucose concentration (%)			
	1.0	2.0	3.0	4.0
10 : 1	13.2[b]	6.4	8.4	6.6
20 : 1	18.6	13.4	12.0	8.4
30 : 1	15.4	14.4	10.0	10.6
40 : 1	16.6	16.4	14.6	13.2

[a] This was made of $MgSO_4 \cdot 7H_2O$ 0.05g, KH_2PO_4 0.46g, K_2HPO_4 1.0g, thiamine-HCl 120 μg, agar 20g, and distilled water 1000 m*l*.
[b] The colony diameter (mm) was measured after 15 days of incubation.
[c] The ratios of $NaNO_3$ versus D-glucose were adjusted to the rate of 10 : 1, 20 : 1, 30 : 1, and 40 : 1, respectively.

Table 6. Mycelial growth of *Sparassis crispa* in the basal medium[a] with different organic acids

Organic acid[e]	Colony diameter[b] (mm)	
Succinic acid	11.2 bc[c]	SC[d]
Gluconic acid	14.8 ab	SC
Citric acid	11.0 bc	C
Fumaric acid	15.8 a	SC
Formic acid	0 e	-
Lactic acid	15.8 a	C
Maleic acid	6.0 d	T
Propionic acid	0 e	-
Oxalic acid	8.2 cd	C
Control	15.5 a	SC

[a] This was composed of $MgSO_4 \cdot 7H_2O$ 0.05g, KH_2PO_4 0.46g, K_2HPO_4 1.0g, thiamine-HCl 120 μg, agar 20g, and distilled water 1000 m*l*.

[b] The colony diameter was measured after 15 days of incubation.

[c] The different letters are significantly different at p=0.01 according to Duncan's new multiple range test.

[d] Mycelial density: C, Compact; SC, Somewhat compact; T, Thin

[e] Each organic acid was added at the rate of 0.1% (w/v).

ture media which were supplemented with formic acid or propionic acid. Chi *et al.* (1996) clarified that *Phellinus linteus* couldn't exhibit a mycelial growth on the culture media containing propionic acid. It was considered that our result was similar to that of Chi *et al.* (1996). It was observed that most of 9 different organic acids were unsuitable for a favorable growth of *S. crispa*.

Based on the obtained basic data, the next study will be focused on the possibility that *S. crispa* can produce a lot of its fruiting bodies on the culture media. Sooner or late, it will be possible to develop a new culture medium suitable for mass production of *S. crispa*.

적 요

꽃송이 버섯의 균사생장은 25°C의 온도와 pH 4의 조건에서 가장 양호하였으며, Hamada 배지에서 가장 조밀한 생장을 나타낸 반면, Hoppkins 배지에서 가장 큰 직경생장을 나타내었다. Maltose, Arabinose 및 Mannitol 같은 탄소원은 꽃송이 버섯의 양호한 생장을 촉진하였다. 또한, 질소원의 하나인 Glycine도 양호한 균사생장을 나타내었다. 배지에 탄소원을 1%로 고정시킨후 C/N비를 20 : 1로 하였을 때, 꽃송이 버섯 균사의 생장이 가장 양호하였다. 유기산으로서 Fumaric acid와 Lactic acid의 처리를 하였을때, 꽃송이 버섯의 생장이 좋았다.

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References

李國均. 1986. 食用菌 栽培技術. 中國 延邊 人民出版社. p.1-366.

劉波. 1978. 中國 葯用眞菌. 中國 山西省 人民出版社. p.1-302.

壓司 當. 1996. マイタケ栽倍から加工 賣り方まで. 農山漁村文化協會. p.1-166.

Chang, H. Y., Cha, D. Y., Kang, A. S., Hong, I. P., Kim, K. P., Seok, S. J., Ryu, Y. J. and Sung, J. M. 1995. Cultural characteristics of *Fomitella fraxinea* (Fr.) *Imaz. Kor. J. Mycol.* **23**(3): 238-245.

Chi, J. H., Ha, T. M., Kim, Y. H. and Rho, Y. D. 1996. Studies on the main factors affecting the mycelial growth of *Phellinus linteus*. *Kor. J. Mycol.* **24**(3): 214-222.

Hong, J. S., Choi, Y. H. and Yoon, S. E. 1986. Studies on the cellulolytic enzymes produced by *Ganoderma lucidum* in synthetic media. *Kor. J. Mycol.* **14**(2): 121-130.

Igarashi, T. and Takeuchi, K. 1985. Decay damage to planted forest of Japanese larchi by wood-destroying fungi in the Tomakomai Experiment Forest of Hokkaido University. *Res. Bull. of Colle. Exper. For., Hokkaido Univ.* **42**(4): 837-847.

Kang, A. S., Cha, D. Y., Hong, I. P., Chang, H. Y. and Yu, S. H. 1994. Studies of cultural condition on the mycelial vegetative growth in *Naematoloma sublateritium* (Fr.) Karst. *Kor.*

J. Mycol. **22**(2): 153-159.

Kim, H. J. 1991. Stand conditions influencing the occurrence of butt-rot disease of larch. *Res. Rep. Inst.* **42**: 155-162.

Kim, H. K., Park, J. S., Cha, D. Y., Kim, Y. S. and Moon, B. J. 1994. Studies on the artificial cultivation of *Lentinus lepideus* (Fr. exFr.) Fr. *Kor. J. Mycol.* **22**(2): 145-152.

Lee, J. Y. 1988. Colored Korean mushrooms. p.1-166. Academic Press. Seoul.

Mao, X. I. and Jiang, C. P. 1993. Economic macrofungi of Tibet. Beijing Science and Technology Press. p.1-651. Beijing.

Park, W. M., Kim, G. H. and Hyeon, J. O. 1995. New synthetic medium for growth of mycelium of *Pleurotus species. Kor. J. Mycol.* **23**(3): 275-283.

Phillips, S. L. and Burdekin, D. A. 1982. Disease of Forest and Ornamental Trees. London: Macmillan.

Song, C. H. and Cho, K. Y. 1987. A synthetic medium for the production of submerged cultures of *Lentinus edodes. Mycologia* **79**(6): 866-876.

Sung, J. M., Kim, C. H., Yang, G. J., Lee, H. J. and Kim, Y. S. 1993. Studies on distribution and utilization of *Cordyceps militaris* and *C. nutans. Kor. J. Mycol.* **21**(2): 94-105.

Wedekind, E. and Fleischer, K. 1923. Uber die konstitution des Sparassols. *Berichtete der deutschen chemischen Gesellschaft* **56**: 2256-2563.

Woodward, S. L., Sultan, H. Y., Barrett, D. K. and Pearce, R. B. 1993. Two new antifungal metabolites produced by *Sprassis crispa* in culture and in decayed trees. *Journal of General Microbiology* **139**: 153-159.