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Fungus Flora of Paddy Fields in Korea
I. Fungal distribution of paddy fields

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韓國 논 土壤中의 菌類에 關한 研究

I. 菌類의 分布

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ABSTRACT

An investigation of the soil microfungal population in the paddy fields of two locations around Seoul was made at four seasons. By the dilution plate method, a total number of propagules of the microfungi per gram of soil was recorded as 10^4 at the upper layer(0-10 cm depth) followed by the middle (10-20cm depth) and the lower layer (20-30cm depth). The highest number of fungal propagules was 11.0 x 10^4 at the upper layer of the soil collected in autumn. The decreasing tendency of the number of fungal propagules was depend on the increasing depth in paddy fields. Seasonal fluctuation of the fungal population was shown from the highest density of fungal colonies on the plate in autumn season and the lowest one in winter, indicating that the autumn is best season for fungal growth. Generally, the number of the species of *Talaromyces* in the paddy soils was found to be very high, particularly in autumn, while comparatively low in winter. The highest number of the species of *Talaromyces* was 6.5 x 10^4 propagules per gram of soil in Yukkog-dong in autumn and the lowest was 0.5 x 10^4 in Shinwon-dong in winter. It is assumed that these fungi grow well also in the warm to the hot seasons.

INTRODUCTION

The soil microfungal populations in native plant communities have investigated during the two decades. However, a few studies were described on the soil mycoflora of the rice paddy fields under the special environmental conditions. Dutta and Ghosh(3) reported the systematic study on the soil microfungal flora of paddy fields where they have cultivated under typically ecological conditions and cultural practices, as it is the most important crop in Orissa, India. Soil samples were collected during four different stages of cultivation or crop growth; ploughed land stage, puddled soil with transplanted crop, active growth period and the post harvest stage. a total number of 165 fungi were isolated from the soil samples obtained from the paddy fields of two sites along with various stages. Among them, the Deuteromycetous fungi were the most frequently encountered group in the study. Certain of the fungi were found to be ecologically mature and dominant in the paddy fields and were present during all the stages of cultivation or growth of rice plants.

Recently, Yokoyama *et al.* investigated the soil microfungi colonizing in the paddy fields of the four selected locations in Osaka, Japan, during a period of more than two years. Although the numbers of colony counted usually varied from plot at every sampling time during three years, there was no marked tendency of the fungal population in a given location.

This paper describes first the population of the soil microfungi in the paddy fields around Seoul, Korea and secondly its seasonal fluctuation together with vertical distribution in the paddy soil.

MATERIALS and METHODS

Sampling sites :

In this experiment, authors have selected two sites of rice paddy fields around Seoul, Korea.

Seoul is located at the center of Korea, between 126° 45′ to 127° 10′ North and 37°25′ to 37°42′ East and surrounded by two mountains lied on north and south, with Han River which is flown down through the center of Seoul. There are typical four seasons in Seoul district area, specially rainy season, wet summer, usually from the middle of July to the end of July, but including cold and dry winter season.

Two different sites were chosen: Shinwon-dong in the south east Seoul and Yukkog-dong, Buchun city in the south west Seoul, representing a soil pH of 6.6 and 6.9, respectively.

These fields have been continuously cultivated with the rice plants. In the winter season, it is very cold and the soil is frozen up to about 90cm depth.

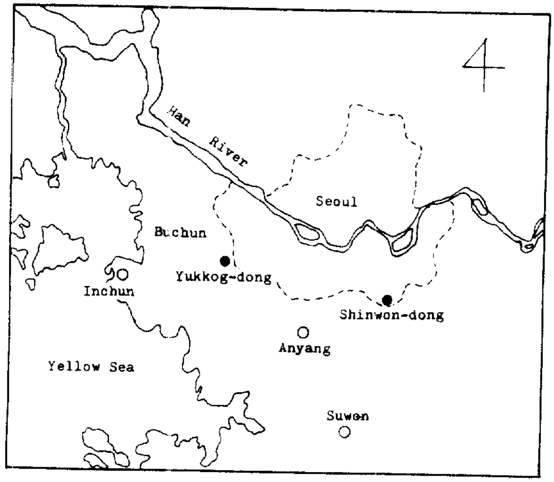

Fig. 1. Map of Seoul prefecture indicating the sites of two selected rice paddy fields

Sampling of soils :

Soil samples were obtained from each plots every three months using a sterile stainless sampler, 70cm long, 28mm wide in inner diameter, with a longitudinal slit of 54cm long and 11mm wide which opens 2cm above the pointed lower edge.

Soil samples for mycological analysis were obtained by pushing sterile samplers vertically into the soil. The soil samples were in different depth from the surface of the paddy field. A soil sample was taken from the slit with a sterile stainless spatula at three different depths: the upper layer (5cm), the middle layer (15cm) and the lower layer (25cm). The schematic profile of soil sampling was shown in Fig. 2. Soil samples were collected during four different stages of cultivation and crop growth; the rest stage of cultivation in spring time (April 25,1980), the active growth period (July 25, 1980), the harvest season (October 25, 1980) and the post harvest season (January 25, 1981).

During the first stage, the land, the temperature and moisture conditions are more or less favourable for the fungal growth. The next stage is presence of abundant water to be sufferred

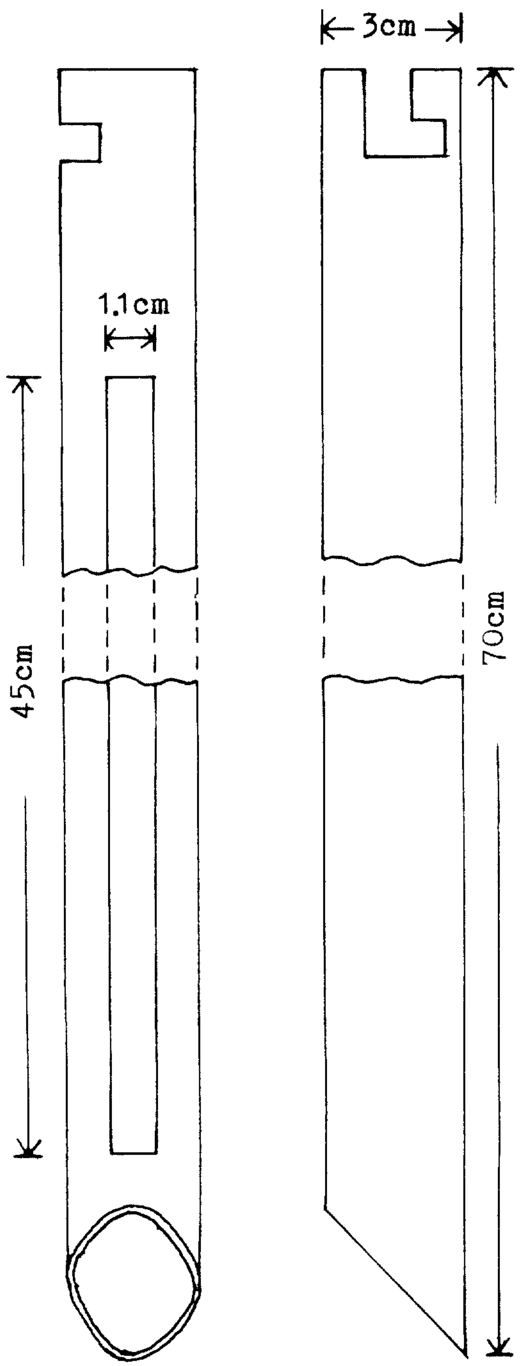

Fig. Diagram of soil sampler

from anaerobic condition for fungal propagation. In the active growth period of plants in autumn season, rice plants grow rapidly, their root exudates are reported to be more condition, and the water content of soil gradually tend to dry up to be suitable moisture. During the last stage of winter season, the post harvest period, is severe cold temperature, sometimes to be snowy or freezen.

Isolation and identification of fungi :

The each culture medium for the isolation of fungi was malt extract-yeast extract agar (MYA) containing tetracycline (50ug/ml) to prevent bacterial development. The medium of MYA was composed of malt extract 0.3g, yeast extract 0.3g, peptone 0.5g, glucose 1.0g, tetracycline 5mg, agar 1.5g and distilled water up to 100ml.

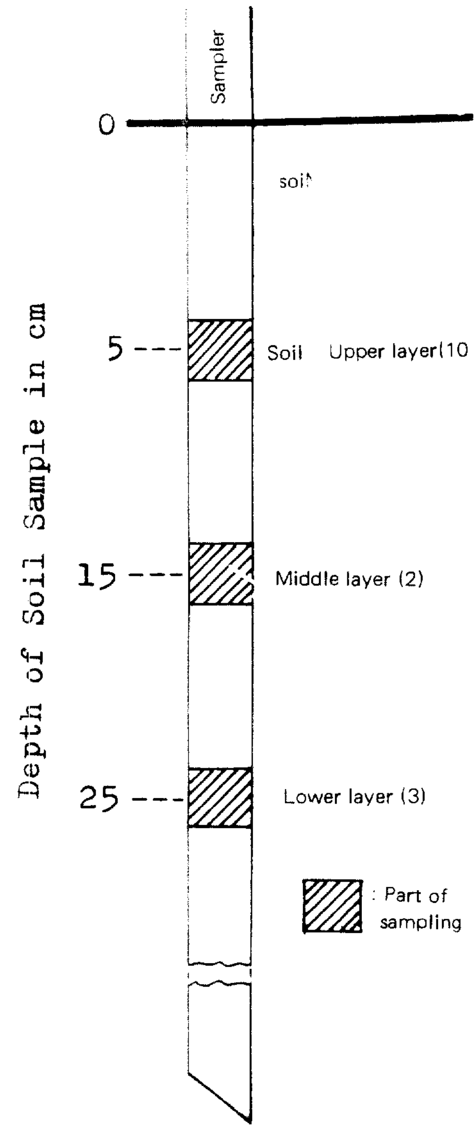

Fig.3. Schematic profile of soil sampling

The isolated fungi from the plates were transferred on a malt extract agar slant at 24°C. The identification of each fungus was carried out on Czapek solution agar, malt extract agar and potato sucrose agar.

Dilution plate method :

Three soil suspensions were different in their soil amounts; one gram of soil from the upper layer, two grams from the middle layer and three grams from the lower layer, respectively.

Each soil sample was added to a sterilized water, 5ml, in test tube and shaked throughly. These aliquots are referred to as soil suspensions henceforward and also used for dilution methods. Plate cultures were consisted of soil suspensions in a sterile water diluted to 1:10 or 1:100, referring to the 10 fold dilution method or 100 fold dilution method, respectively. Five plates for each dilution per each soil samples were made by spreading each 0.1ml of soil dilution suspensions and incubated at 24°C until colonies were developed.

Appearing the colonies, the incubated plates were observed and counted under a dissecting microscope after 3 to 4 days. For monospore culture, only one colony of same cultural characteristics was taken to isolate carefully, but prevent from duplicates strains for each species as possible.

RESULTS

Soil samples were collected from two sites, Yukkog-dong in Buchun city and Shinwon-dong in south east of Seoul, at three month intervals from April 25, 1980 to January 25, 1981. These

Table 1. Number of fungal propagules* per gram of soil from Yukkog-dong during four seasons

Depth in cm	season / ** Dilution / Number of Plate	Spring		Summer		Autumn		Winter	
		10	100	10	100	10	100	10	100
	1	4.8	0.0	4.5	5.0	7.8	9.0	3.5	5.0
	2	4.0	7.5	3.5	7.5	8.0	9.0	4.3	2.5
5	3	3.5	5.0	4.3	5.0	8.5	7.0	3.0	5.0
(Upper	4	3.8	5.0	4.3	2.5	6.9	8.5	2.5	7.0
layer)	5	5.5	15.0	4.0	5.0	6.8	9.0	3.3	0.0
	Average	4.3	6.5	4.1	5.0	7.6	8.5	3.3	4.0
	1	3.9	3.8	2.3	2.5	5.1	6.3	2.5	3.8
	2	4.1	5.0	2.5	1.3	6.5	8.8	4.5	5.0
15	3	3.9	3.8	1.5	1.3	5.6	7.5	2.3	2.5
(Mid-	4	3.5	6.2	1.9	3.8	6.0	5.0	4.2	3.7
dle	5	3.6	2.5	2.4	2.5	5.3	8.8	2.0	1.3
layer)	Average	3.8	4.3	2.1	2.3	5.7	6.9	3.0	3.3
	1	2.3	4.2	1.2	2.5	2.5	3.5	1.2	1.7
25	2	1.6	4.2	1.2	1.6	3.4	3.1	0.8	2.5
(Low-	3	2.1	2.4	0.4	2.4	2.6	4.9	1.4	1.7
er	4	2.5	1.6	1.6	0.8	3.8	1.6	1.3	0.0
layer)	5	2.4	1.6	1.5	2.5	3.4	3.3	1.3	2.0
	Average	2.2	2.8	1.2	2.0	3.2	3.4	1.2	1.7

* Fungal propagules were counted on the plate culture which was made from soil suspension diluted to 1:10 or 1:100 ($\times 10^4$).

** Soil suspension was diluted to 1:10 (10 fold dilution) or 1:100 (100 fold dilution), respectively.

Table 2. Number of fungal propagules* per gram of soil from Shinwon-dong during four seasons

Depth in cm	season / **Dilution / Number of plate	Spring 10	Spring 100	Summer 10	Summer 100	Autumn 10	Autumn 100	Winter 10	Winter 100
5 (Upper layer)	1	6.0	10.0	6.75	7.5	8.5	12.5	8.8	5.0
	2	5.3	2.5	5.3	5.0	9.3	15.0	3.0	7.5
	3	4.3	7.5	6.0	10.0	8.7	10.0	9.0	12.5
	4	5.3	2.5	4.8	12.5	8.5	10.0	6.0	7.5
	5	3.8	12.5	4.7	2.5	6.0	7.5	9.0	7.5
	Average	4.9	7.0	5.5	7.0	8.2	11.0	7.4	6.5
15 (Middle layer)	1	2.4	6.3	4.3	6.3	10.4	3.0	2.1	5.0
	2	5.1	5.0	4.0	9.5	12.6	7.0	2.3	3.8
	3	4.5	3.8	4.0	6.3	11.7	10.5	1.9	2.5
	4	3.4	5.0	2.5	2.5	10.3	9.0	2.5	6.3
	5	3.6	5.0	2.6	2.5	12.1	11.3	2.1	5.0
	Average	3.8	5.3	3.5	5.3	11.4	8.5	2.2	4.5
25 (Lower layer)	1	3.7	3.3	2.9	6.7	6.3	7.5	1.7	4.2
	2	3.3	0.0	3.4	4.2	5.7	5.8	1.6	3.3
	3	3.4	4.2	3.5	3.3	6.7	6.7	1.3	1.7
	4	4.2	5.0	3.3	2.5	5.5	5.0	1.8	5.0
	5	3.3	5.8	3.4	1.7	5.5	9.2	1.4	4.2
	Average	3.6	3.6	3.3	3.6	5.9	6.8	1.6	3.8

* Fungal propagules were counted on the plate culture which was made from soil suspension diluted to 1:10 or 1:100 ($\times 10^4$).

** Soil suspension was diluted to 1:10 (10 fold dilution) or 1:100 (100 fold dilution), respectively.

four stages are not only significant as they include distinct growth phases of the rice plant but also the paddy field soil has distinct ecological changes due to the temperatures, water contents in soil, and cultural practices in a year.

All samples used in this present investigation were subjected to the dilution plate method. By using the soil suspension diluted to 1:10 (10 fold dilution method) and 1:100 (100 fold dilution method), the numbers of the fungal colonies were encountered when the fungal colonies were developed.

However, it is impossible to examine in detail all the fungal colonies which grow on the dilution plates. The authors investigated the comparison of fungal propagules per gram of soil with different experimental sites, depths, and seasons in this present experiment. A number of unidentified species were also encounted, but with the exception of one extremely interesting *Talaromyces* spp., therefore, there are not discussed in this present paper.

The results obtained from soils at the paddy fields of two sites were shown in Fig. 4 and 5. It was found that the paddy field soils of Shinwon-dong and Yukkog-dong in the autumn of 1980 have shown to be the highest fungal populations in this experiment using 100 fold dilution method, representing 11.0×10^4 and 8.5×10^4 in average numbers of the fungal propagules per gram of the upper layer soil, respectively. On the other hand, the lowest fungal count of the lower layer of Yukkog-dong was recorded as average number, 1.7×10^4 in winter season and also it was showed as 3.6×10^4 in the spring and the summer of 1980 at Shinwon-dong (Table 1 and

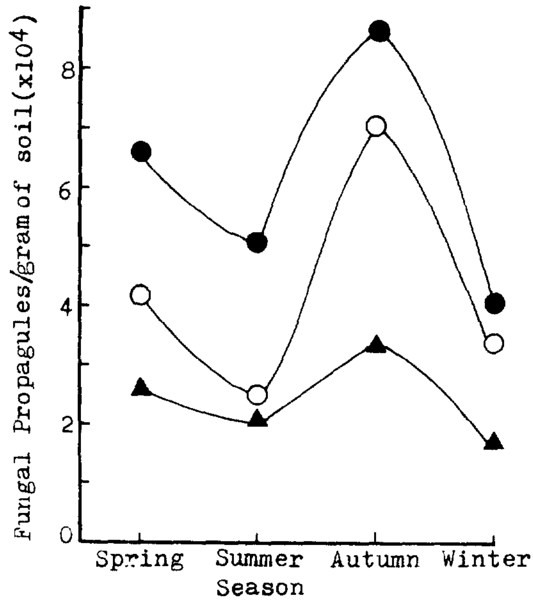

Fig. 4 Seasonal fluctuation of fungal propagules at Yukkog-dong by the 100 fold dilution method. Soil sampling parts were the upper layer (●—●), the middle layer (○—○) and the lower layer (▲—▲).

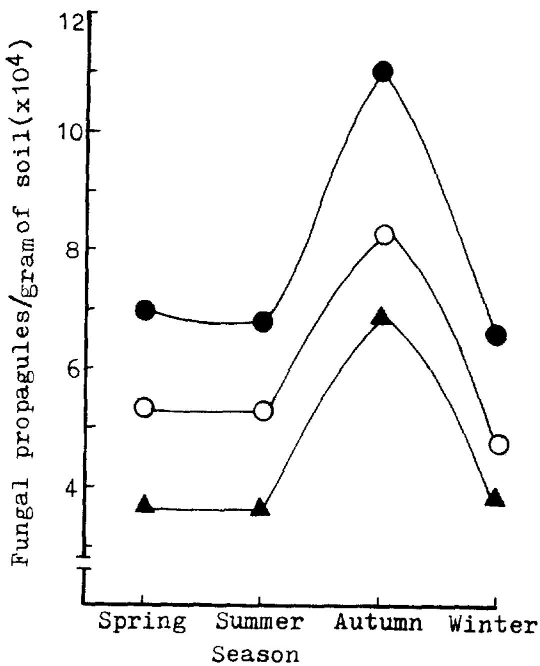

Fig. 5. Seasonal fluctuation of fungal propagules at Shinwon-dong by the 100 fold dilution method. Soil sampling parts were the upper layer (●—●), the middle layer (○—○) and the lower layer (▲—▲).

2)

Vertical distribution of fungal propagules : Comparing with the vertical distribution of the fungal population at two sites, the number of the fungal propagules showed on the dilution plates was the highest in the soil of the upper layer (5cm) followed by the middle layer (15cm) and the lower layer (25cm).

In autumn season at Shinwon-dong site, the number of fungal population of the upper soil sample was of the highest value, while that of the lower layer the lowest.

At the upper layer in each field, more than 10^4 propagules per gram of soil were obtained as well as at the middle or lower layer.

Generally, the decreasing tendency of the number of fungal propagules per gram of soil with the increase of depth was proved in the paddy field of two sites around Seoul. This tendency agreed with all four seasons of different soil environments eventhough the paddy field has special ecological conditions.

As described above, a comparison between the species of fungi isolated from two plots showed almost similar tendency in the vertical distribution of paddy soil fungi.

Seasonal fluctuation of fungal propagules : The result obtained from soils of two sites showed the seasonal fluctuations in numbers of the fungal propagules appeared on the dilution plates. In general, the fluctuation in numbers of the fungal populations seems to be parallel to all three layers. In any cases at two plots, there was a particular tendency; the highest density of fungal colonies on the plate in autumn season but the lowest in winter and summer season.

This low tendency of the fungal population in summer season seems to be interpreted that the large amount of water in paddy fields at two sites do not provide good conditions for fungal growth. On the other hand, the highest tendency of the fungal population in autumn season is assumed that the autumn provide not only good temperature for fungal propagation, but also suitable water content in paddy soil, associated with the growth of rice plants. In contrast to summer season, the fungal population in soil at two sites in winter showed the lowest value than in any other seasons. In winter season the land is frozen up to 90cm depth and it is not good environment for fungal propagation.

Dutta and Ghosh(4) had pointed out a distinct variation in the fungal flora from season to season and with different stages of cultivation including crop growth. This distinct variation of fungal flora is good agreement with our results in this investigation.

Distribution of *Talaromyces* spp. :

In order to confirm the presence of a difference between the number of *Talaromyces* spp. per gram of soil in autumn and in winter, the present authors extended our work by detecting the *Talaromyces* population during autumn and winter seasons with the exception of other seasons.

By the dilution plate method on petridish culture, total populations of *Talaromyces* spp. at two sites were examined in the upper, the middle and the lower layers.

The results of these investigations are summarized in Table 3 and 4. It was also found in the present work that the distribution of predominant species, *Talaromyces* spp. reveals the difference between autumn and winter seasons.

In Table 3, the total number of *Talaromyces* spp. per gram of soil at Yukkog-dong in autumn appeared to be the highest average value (6.5 x 10^4) and the lowest 0.2 x 10^4 in winter. In case

Table 3. Number of *Talaromyces* spp. per gram of Soil in Yukkog-dong

Depth in cm	Season / *Dilution / Number of plate	Autumn 10	Autumn 100	Winter 10	Winter 100
5 (Upper layer)	1	3.50	7.50	0.25	0.00
	2	3.50	7.50	0.25	0.00
	3	3.00	5.00	0.50	0.00
	4	2.50	7.50	0.75	2.50
	5	1.50	7.50	0.75	2.50
	Average	2.90	6.50	0.50	1.00
15 (Middle layer)	1	6.25	5.00	0.36	1.25
	2	6.25	7.50	0.00	0.00
	3	8.00	3.75	0.50	0.00
	4	2.88	3.75	0.00	0.00
	5	0.00	5.00	0.00	0.00
	Average	4.68	5.00	0.20	0.25
25 (Lower layer)	1	0.25	3.33	0.25	0.00
	2	0.17	2.50	0.58	1.67
	3	0.33	3.33	0.50	0.83
	4	0.08	0.83	0.25	0.00
	5	0.17	1.67	0.58	0.83
	Average	0.20	2.33	0.43	0.67

*All methods of this experiment were the same as Table 1 and 2

Table 4. Number of *Talaromyces* spp. per gram of soil in Shinwon-dong

Depth in cm	Season / *Dilution / Number of plate	Autumn 10	Autumn 100	Winter 10	Winter 100
	1	0.50	2.50	0.25	2.50
	2	0.50	10.00	0.50	5.00
5	3	0.25	2.50	0.25	0.00
(Upper	4	1.00	10.00	0.25	2.50
layer)	5	0.00	2.50	0.25	2.50
	Average	0.45	5.50	3.00	2.50
	1	1.13	2.50	0.25	0.00
	2	0.75	3.75	0.50	0.00
15	3	1.25	10.00	0.38	2.50
(Middle	4	0.75	1.25	0.63	5.00
layer)	5	1.63	2.50	0.75	0.00
	Average	1.10	4.00	0.50	1.50
	1	1.00	1.67	0.42	2.50
	2	1.33	1.67	1.33	0.83
25	3	1.42	1.67	0.25	2.50
(Lower	4	1.58	0.83	1.25	1 .67
layer)	5	1.33	0.83	1.00	2.50
	Average	1.33	1.33	0.85	1.17

*All methods of this experiment were the same as Table 1 and 2

of Shinwon-dong, as shown in Table 4, the total number of *Talaromyces* spp. per gram of soil was found to be the highest average value (5.5×10^4) in autumn while the lowest 0.5×10^4 in winter season.

In general, the number of *Talaromyces* spp. in soil samples in autumn was higher than that of in winter at two sites. Comparing with Table 1 and 2, it was found that the tendency of the distribution of *Talaromyces* spp., was correlated to the distribution of total soil fungi and *Talaromyces* spp. was considered as one of the most dominant fungi in paddy field soils.

As shown in Table 3 and 4, it is shown that the numbers of fungal propagules per gram of soil using 100 fold dilution method was generally higher than those of the fungal population using 10 fold dilution method.

DISCUSSION

The present isolates obtained from the soil samples at two stations was summarized here and informed to the microfungal flora paddy fields.

It was reported by Yokoyama *et al.* that, although the counts of the fungal numbers varied from plot to plot in given field, the calculated numbers of the fungal propagules per gram of soil were comparatively constant if the order in numbers of fungal propagules is taking into account. Moreover, it was confirmed that these fungi could constantly be isolated at the given sites throughout the investigation.

The present study was carried out to investigate the microfungal population and variation of the paddy soil fungi during one year only. However, the fact obtained from the above mentioned result would suggest that the filamentous fungi isolated from two sites at different seasons and depths in this work can be reisolated constantly at the same site and in the same season in any year.

It was pointed out by Yokoyama *et al.* that the soil temperature and the numbers of fungal propagules detected by the dilution plate method showed a positive correlation. Significant decrease in fungal counts was coincided with the highest temperature of the soil.

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요 약

서울근교 역곡동과 신원동의 논토양 시료들 중에서 계절별 균류의 수직분포를 조사하였다.

평판희석법을 사용하여 토양 1g중 총균류의 평균수는 상층부(5 cm 깊이)에서 10^4 이상을 나타내었으며 그다음이 중층부(15 cm 깊이), 하층부(25 cm 깊이)의 순서이었다. 가장 많은 총 균류의 분포는 가을토양의 상층부로 평균 11×10^4이었다.

또한 균류의 수는 논 토양의 깊이가 깊어질 수록 감소하였다. 균 분포의 계절적 변이는 겨울에는 낮으나 가을에는 가장 높았으며, 이 사실로 가을이 균 생장에 가장 좋은 환경조건임을 알 수 있었다.

일반적으로, 논 토양에서의 *Talaromyces*의 수는 겨울에 적은 반면에 특히 가을에 현저하게 많으며, 역곡동의 가을 시료의 경우에는 토양 g당 6.5×10^4 propagules로 최고수를 나타냈고, 신원동의 겨울시료가 0.5×10^4으로 최저의 수를 나타내었다.

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