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Seasonal and Spatial Distribution of *Trichoderma* species in Forest Soils of Mt. Geryongsan

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계룡산 산림토양내의 수종 *Trichoderma* spp.의 분포 특성에 관하여

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ABSTRCT

Seasonal and spatial variations in propagule numbers of *Trichoderma* species were investigated every other month for one year in deciduous and coniferous forest soils and evaluated the relationships of *Trichoderma* spp. populations to soil environmental factors.

The total population of *Trichoderma* spp. increased until summer and then declined until winter. The yearly mean frequency of *Trichoderma* spp. exceeded 1.4% of total fungal propagules in two sites. Decreases of absolute and relative propagule numbers of *Trichoderma* spp. with increasing soil depth were found and variation in *Trichoderma* spp. propagules caused by differences in soil depth (0~50cm) was greater than that caused by differences in sampling time.

The most common species occurring in two sites was *T. viride*, followed by *T. polysporum*, *T. koningii*, and *T. hamatum*. Individual species of *Trichoderma* showed different abundance trend in accordance with sampling time. *T. viride* was dorminant from spring to autumn, while *T. polysporum* dominated over the other species in winter. Variations in propagule number of *Trichoderma* spp. were principally mediated by the actions of biotic environmental factors rather than by the direct effects of abiotic factors. In multiple-regression analyses, 48% of the total vaiation in *Trichoderma* spp. propagules in deciduous site could be accounted for by total fungal propagules and soil CMCase activity. In coniferous site, 65% of total variation could be accounted for by total fungal and bacterial propagules, moisture content and organic carbon content.

INTRODUCTION

In recent years, interests in the role of *Trichoderma* species are increasing because of their known ability to produce volatile and nonvolatile antibiotics against a range of microorganisms (Dennis *et al.*, 1971; Fujiwara *et al.*, 1982; Okuda *et al.*, 1982) and to synthesize cellulase components and any other enzymes which are necessary for the degradation of natural polymers (Rosenberg, 1978; Theodorou *et al.*, 1980; Sandhu *et al.*, 1980). In addition, the parasitism of soil-born plant pathogenic fungi by *Trichoderma* species has been reported and indicated that the antagonistic properties of *Trichoderma* spp. might be the most promising biological control agents (Kelley *et al.*, 1976; Chet *et al.*, 1981).

Despite of their powerful influences on soil

ecosystem, knowledge of the ecological characteristics of individual species of *Trichoderma* is comparatively sparse. Danielson *et al.* (1974a) investigated the general trends with regard to the distribution of species aggregates of *Trichoderma* in relation to geographic and climatic conditions and concluded that *T. koningii* and *T. hamatum* were the most common species in all the climatic regions and *T. viride* and *T. polysporum* were largely restricted to cool temperature regions, whereas *T.harzianum* was characteristic of warm climates. Similar phenomenon was observed by Widden *et al.* (1980) in spruce-forest soil in southern Quebec. Meanwhile, Nelson (1982) observed the occurrence of *Trichoderma* spp. at depth of 100cm in soil supporting a Douglas-fir stand in western Oregon.

Informations on the distribution patterns of *Trichoderma* species in natural soil ecosystem should be available not only for the selection of suitable species as a biocontrol agent under specific circumstances but also for the application of its role in the decomposition of plant polymers.

In earlier investigation on the distribution of *Trichoderma* species in forest soils of Mt. Geryongsan (Rhee *et al.*, 1982), five species of *Trichoderma* propagules were isolated and the genus was found to be a major element, especially in L and F horizon commonly in hardwood and conifer soils.

As an extention of the previous work, seasonal variations of each species of *Trichoderma* propagules in deciduous and coniferous forest soils were investigated in connection with different soil layer representing habitat alterations. Moreover, in order to undarstand the environmental factors influencing the occurrence of *Trichoderma* spp., the relationships of this genus to biotic and abiotic soil environmental factors were evaluated.

MATERIALS AND METHODS

The study areas

Laboratory experiments were carried out with soils sampled from two distinct plant communities, deciduous(*Quercus mongolia*) and coniferous (*Chamaecyparis obtusa*) forest communities in Mt. Geryongsan, middle of Korea (36°21'N, 127°15'E). All the soil samples were collected from each of the two stands bimonthly, from June 1982 through June 1983, at five randomly selected points within each stand. To estimate the vertical distribution of *Trichoderma* species, optional layers were established as D1 (0-10cm depth), D2 (10-20cm), D3 (20-30cm), D4 (30-40cm) and D5 (40-50cm). Approximately 500g of samples were collected from each layer of the two sites. After screening through a sieve (mesh size, 2mm) samples were stored in polyethylene bags at 4°C. The surface horizon of deciduous site was a dark brown (10YR4/3) silt loam and that of coniferous site was a brown (10YR5/3) sandy clay loam.

Chemical analysis

All analytical results were calculated on the basis of oven-dry (100°C) weight of soil and at a minimum, analyses were made in duplicate. The moisture content was determined from the loss in weight after drying at 100°C to a constant weight. The soil pH was estimated in a slurry with water (1 part soil to 2.5 parts water). Soil organic carbon content was measured by Walkley-Black method (Jacson, 1958). Total Kjeldahl nitrogen contents detected by the molybdate blue method were determined colorimetrically.

Estimation of microfungal populations

Microfungal population sizes were estimated by the serial dilution plate method, each of the dilutions was plated out in triplicate using 0.1ml samples. The medium used for the isolation of

microfungi was modified Czapec-Dox agar (20g of glucose, 2.8g of $(NH_4)_2SO_4$, 1g of $NaNO_3$, 1g of K_2HPO_4, 0.5g of $MgSO_4 \cdot 7H_2O$, 0.5g of KCl, 0.01g of $FeSO_4 \cdot 7H_2O$, 5g of oxgall, 1.0g of chloramphenicol, 20g of agar and 1l of distilled water). The plates were incubated under aerobic conditions at 26± 1°C for 5-7 days.

Statistical analysis

Simple statistics and coefficient of variation (CV%) were computed to compare variabilities in different layers and different sampling times. The relationship between *Trichoderma* propagules and environmental factor was established by calculating simple linear correlation coefficient. Multiple-regression analysis was used to explain the changes in *Trichoderma* spp. propagules in terms of the changes in environmental parameters.

Assay of soil CMCase

After screening through a 2mm sieve, a 5g air dried soil sample was mixed thoroughly with 10ml of 0.1M acetate buffer (pH 5.4) and 10ml of 1% carboxymethylcellulose. The mixture was then incubated for 20hrs at 40°C. At the end of this reaction period approximately 50ml of distilled water were added and filtrated. The reducing sugar content was then determined by Somogyi-Nelson method.

RESULT AND DISCUSSION

Characteristics of soils

Some soil environmental parameters which influence the changes in microbial populations were measured bimonthly (Table 1). Compared with the coniferous site, the deciduous site showed low pH level and though, there were no significant changes of soil pH with respect to sampling time and soil depth, gradual decreases of pH level from winter to summer were commonly observed in two sites. Although air temperatures exceeded 35°C in summer and dropped to −15°C in winter, the soil temperatures of two forest soils fluctuated between a high of 22°C in August and a low of −5°C in February. The moisture contents of deciduous and coniferous site fluctuated around 40% and 30% of the dry weight for most of the year, respectively. Organic carbon content in deciduous site was approximately two times higher than coniferous site and tended to be highest in February. Remarkable variation in total nitrogen content was not found throughout a

Table 1. Soil properties of two different forest-stands. (Mean ± Standard Error)

Site \ Month	JUN	AUG	OCT	DEC	FEB	APR
Deciduous Forest Stand						
pH	4.2±0.14	4.5±0.13	4.36±0.08	5.12±0.08	5.14±0.10	5.0±0.05
Temperature (C)	18.40±0.33	18.7±0.37	18.1±0.29	1.90±0.86	0.30±1.45	11.6±0.66
Moisture content (%)	37.36±2.54	42.8±1.30	31.14±1.16	36.6±0.65	43.14±1.97	46.0±1.23
Organic C (%)	7.46±1.32	4.536±1.68	6.03±1.80	8.896±1.26	14.33±1.69	9.18±0.91
Total N (%)	0.274±0.04	0.25±0.05	0.25±0.03	0.23±0.03	0.34±0.03	0.234±0.04
Coniferous Forest Stand						
pH	4.98±0.09	4.96±0.07	4.90±0.06	5.10±0.09	5.32±0.06	5.04±0.06
Temperature (C)	18.5±0.32	21.7±0.26	20.0±0.16	0.30±0.89	−0.40±1.57	10.4±0.24
Moisture content (%)	24.78±1.79	17.68±0.78	24.88±1.81	17.68±0.55	18.66±2.33	25.16±5.54
Organic C (%)	3.61±1.01	3.45±1.33	4.57±1.50	2.38±0.61	5.38±1.75	2.82±0.51
Total N (%)	0.22±0.03	0.20±0.02	0.21±0.01	0.14±0.02	0.24±0.02	0.16±0.03

Table 2. Seasonal distribution of *Trichoderma* spp. in deciduous and coniferous forest soil.

Deciduous forest soil			(Year) Month	Coniferous forest soil		
Population size of *Trichoderma* spp. ($\times 10^2$/g)	Relative pop. size of *Trichoderma* spp. (%)	CV** (%)		Population size of *Trichoderma* spp. ($\times 10^2$/g)	Relative pop. size of *Trichoderma* spp. (%)	CV** (%)
10.6±2.5*	1.18	52.3	1982 JUN	7.4±1.5	1.25	47.2
10.8±2.3	1.79	47.4	AUG	6.0±1.3	2.13	48.1
10.4±2.3	1.09	51.2	OCT	6.8±1.2	1.36	39.7
6.0±1.2	1.25	44.7	DEC	3.0±0.6	0.92	42.3
5.3±1.2	1.39	50.6	1983 FEB	3.8±0.8	1.14	48.4
9.2±2.0	1.90	48.8	APR	5.8±1.3	1.86	50.3

*: mean±standard error

**: coefficient of variation between different soil depth

year but the highest values were shown in February in common with those of organic carbon. In contrast, sharp decreases in contents of organic carbon and total nitrogen with increasing soil depth, especially between D1(0-10cm depth) and D2 (10-20cm depth) were observed.

Seasonal distribution of *Trichoderma* spp.

Variations in propagule number of *Trichoderma* spp. caused by differences in sampling time were examined. As shown in Table 2, the propagule numbers of *Trichoderma* spp. and ratios of *Trichoderma* spp. to total fungi differed considerably between the sampling times. Total population of *Trichoderma* spp. in the two sites increased until June (in deciduous site) or August (in coniferous site) and then declined until February (in deciduous site) or December (in coniferous site). The seasonal trend of propagule numbers of *Trichoderma* spp. is in agreement with the results of other investigators. Widden *et al.* (1980) found the increase of *Trichoderma* spp. propagules until July in spruce forest soil and Nelson (1982) also demonstrated the most frequent occurrence of *Trichoderma* spp. in August in Douglas-fir soil.

Although more abundant propagules of *Trichoderma* spp. occurred in deciduous site, there were no distinct differences in relative population size of *Trichoderma* spp. (percent of total fungal propagules) between the two soil stands. Relative populalion sizes of *Trichoderma* spp. in deciduous site were accounted for 1.09-1.90% (mean: 1.42%) and those of coniferous site were 0.92-2.13% (mean: 1.44%). The data accorded with the results obtained by Widden (1979) from the some deciduous and coniferous sites located in southern Quebec. However, Okada (1938) reported the different ratios (0-83.3%) of *Trichoderma* spp. to all fungi according to various vegetation types. From spruce forest soil in south of Sweden, Söderström (1975) found that *Trichoderma* spp. made up 11% of all isolates. By all accounts it seemed that the enormous discordances in relative population sizes of *Trichoderma* spp. with the different investigations were due to differences in techniques used for the isolation of *Trichoderma*. spp. For example, Nelson (1982) could isolate only one colony of *Trichoderma* spp. in nearly 2000 fungal colonies by dilution plate method, whereas it was isolated at a frequence of 10% from Mueller-Durrell tubes in soil supporting a Douglas-fir stand. In addition, there is no one medium that is entirely used for the isolation of all kinds of soil microorganisms and consequently types of medium used in experiments might be an another main element affecting the relative population sizes

Table 3. Vertical distribution of *Trichoderma* spp. in deciduous and coniferous forest soil.

Deciduous site			Soil depth	Coniferous site		
Pop. size of *Trichoderma** spp. ($\times 10^2$/g)	Relative pop. size of *Trichoderma* spp.(%)	**CV (%)		Pop. size of *Trichoderma** spp. ($\times 10^2$/g)	Relative pop. size of *Trichoderma* spp.(%)	**CV(%)
20.6±2.4	1.85	28.7	D_1(0-10cm)	13.4±1.7	2.07	31.4
10.2±1.1	1.43	26.8	D_2(10-20cm)	6.0±0.7	1.75	29.5
5.9±0.8	1.29	34.4	D_3(20-30cm)	3.6±0.5	1.22	32.4
3.0±0.4	1.28	33.2	D_4(30-40cm)	1.3±0.1	1.03	25.7
2.3±0.3	1.25	35.3	D_5(40-50cm)	1.3±0.1	1.11	27.2

*: mean±standard error

**: coefficient of variation between different sampling time

of *Trichoderma*. spp.

Vertical distribution of *Trichoderma* spp.

Table 3 shows the spatial variations of *Trichoderma* spp. propagules occurred in two forest soils with respect to different soil depth. Generally, decreases of absolute and relative propagule numbers of *Trichoderma* spp. with increasing soil depth were observed commonly in the two sites. A conspicuous reduction occurred especially between D1 and D2 layer. The relative population sizes of *Trichoderma* spp. in D1 layer were approximately eight times greater than those of D5 layer.

Even though it is difficult to explain environmental factors which are probably responsible for the limitation of vertical distribution, the present data indicated that *Trichoderma* spp. propagules were, at least to a depth of 50cm and occurred much more frequently in soil layers high in organic content. A limiting supply of oxygen, especially in poorly aerated soils have been proposed as a factor preventing development of more extensive fungal populations in the deeper soil horizons (Wicklaw *et al.*, 1974; Witkamp, 1971). In contrast, Nelson (1982) isolated *Trichoderma* species to a depth of 100cm and proposed that soil aeration is not a limiting factor but organic content is a major factor in occurrence of *Trichoderma* spp. in deeper soil horizons. In the previous report(Rhee *et al.*, 1982), it was indicated that organic content is not the most important factor despite of the most occurrence of *Trichoderma* spp. propagules in L and F horizons. Further detailed investigations are therefore needed to understand the limiting factor(s) affecting the vertical distribution of *Trichoderma* spp. For a comparison the variations of propagule numbers of *Trichoderma* spp. causing by differences in sampling time and soil depth, tabulations of the mean coefficient of variation (CV%) were also performed on the examined data. As shown in Table 2 and 3, the mean CV values for the variation between different soil depth were 49.2% (in deciduous site) and 46.0% (in coniferous site), which were higher than those for the variation between different sampling time. From these data, it is probably possible to suggest that variation in propagule numbrs of *Trichoderma* spp. caused by differences in soil depth was greater than that caused by differences in sampling time. There are no available data which are comparable with these results, but same greatness of spatial variation in fungal biomass with the variation between different sampling dates were demonstrated by Baath *et al.* (1982).

Species distribution

Isolates of *Trichoderma* spp. were identified to species using Rifai's (1969) key and the bethavior of individual species of *Trichoderma* was compared each other by the observation of

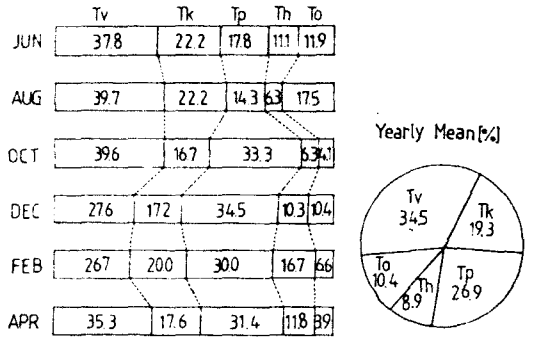

Fig. 1. Occurrence frequency of individual species of *Trichoderma* with respect to sampling time in deciduous site (percent of total *Trichoderma* isolates). Tv: *T. viride*, Tk: *T. koningii*, Tp: *T. polyspoum*, Th: *T. hamatum*, To: other species of *Trichoderma*

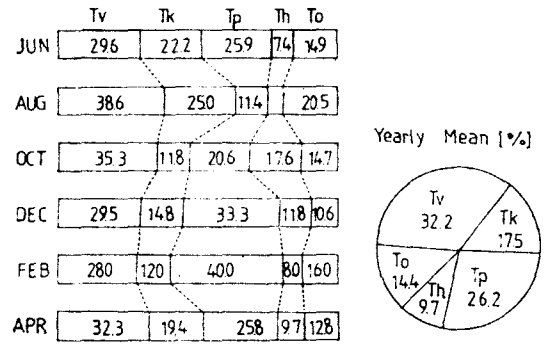

Fig. 2. Occurrence frequency of individual species of *Trichoderma* with respect to sampling time in coniferous site. All abbreviations are the same as Fig. 1.

their occurrence patterns with respect to sampling time.

As presented in Fig. 1 and 2, there were no noteworthy differences in species distribution between deciduous and coniferous site but marked effects of sampling time on the distribution of individual species were observed. Among the identified species, when comparing the yearly mean frequency value, the most common species in two sites was *T. viride*, followed by *T. polysporum*, *T. koningii* and *T. hamatum*, and these 4 species comprised 91.1% and 85.6% of all the *Trichoderma* spp. isolates of deciduous and coniferous site, respectively. In common with the data presented in this study, *T. polysporum* and *T. viride* have been reported as the most common species in a number of forest soils (Söderstrom, 1975; Widden *et al.*, 1980; Nelson, 1982). Furthermore Widden (1979) suggested the statistically significant difference in abundance for *T. polysporum* and *T. hamatum* between deciduous and coniferous site but no differences between the two sites were found in this study.

T. viride was abundant throughout a year and was dorminant from spring to autumn. On the other hand, *T. polysporum* was dominant species in December and February, and decreased to low levels in August. In spite of the overall seasonal effect, *T. koningii* did not show any conspicuous changes in abundance frequency from month to month. *T. hamatum* showed increasing abundance from autumn to winter and tended minimum in August. Up to now, a few investigations have demonstrated the distributions of *Trichoderma* species. Danielson *et al.* (1974a) indicated that *T. viride* and *T. polysporum* were geographically restricted to cooler regions and *T. koningii* and *T. hamatum* were widely distributed. In addition, it was also suggested that species of *Trichoderma* typical of cool geographic regions possessed lower temperature optima and maxima than those from warm climatic regions (Danielson *et al.*, 1974b). These trends were also found by Widden *et al.*(1980), who pointed out that *T. polysporum* was most abundant in the fall and winter, *T. viride* in the spring and fall, and *T. koningii* occurred most frequently in the summer. The data presented here are generally consistant with the those of Widden *et al.*, with the exception of the occurrence of *T. viride* to a large extent in summer. However, strikingly contrasting results were proposed by Martinez *et al.* (1979), who studied microfungal community of an andosols and found the seasonal trends of *T. harzianum* with diminish-

ing abundance from winter to autumn, *T. polysporum* with opposite behavior, and *T. hamatum* with increasing from winter to summer.

These results of the seasonal trend of *Trichoderma* species may be indicating that *Trichoderma* species vary greatly in their ecological and physiological properties, and then show differing ecological niches and responses to the complex environmental factors. In order to obtain more useful informations further studies on their ecological and physiological properties are needed.

Effect of environmental factors

In an attempt to elucidate the variations in the propagule numbers of *Trichoderma* spp. in terms of the changes in the environmental parameters, the relationships of *Trichoderma* spp. density to abiotic and biotic factors were established by calculating simple linear correlation coefficients.

Generally, environmental parameters which are responsible for the distribution of soil microorganisms include climatic factor, physicochemical properties of soil, nutritional factor, and influences of the associated microflora. Among the abiotic parameters, soil temperature and soil pH are obviously major environmental factors governing the growth and activity of the fungi as pointed out for example by Bissett *et al.* (1979), and especially, the tremendous seasonal variation in numbers of fungi can easily be attributed to the effects of temperature. Neverthless, both of soil temperature and pH did not showed any significant correlations ($P<0.05$) with the abundance *Trichoderma* spp. (Table 4). This is in accordance with the results of Lundgren *et al.*(1983), who found that soil temperature or changes in temperature did not influence the bacterial numbers to any detectable degree in soil. Meanwhile, as indicated by many investigators (Lund *et al.*, 1980; Hunt, 1983) as a primary controlling factor,

Table 4. Correlation coefficients (r-values) between the propagule numbers of *Trichoderma* spp. and environmental factors in two forest soils.

VARIABLES	Propagule number of *Trichoderma* spp.	
	Deciduous site	Coniferous site
Soil pH	NS+	NS
Soil temperature	NS	NS
Moisture content	NS	0.610***
Total nitrogen content	0.694***	0.537**
Organic carbon content	0.423*	0.779***
Soil respiration	0.744***	0.726***
Number of total bacteria	0.711***	0.653***
Number of total fungi	0.858***	0.834***
Soil CMCase activity	0.497**	0.648***

NS+ : Not significant
* : Significant at the 0.05 level
** : Significant at the 0.01 level
*** : Significant at the 0.001 level

Table 5. Equations from multiple-regression analyses for the occurrence of *Trichoderma* spp. in two forest soils.

Site	Regression equation	R^2
Deciduous	$Y=3.78+0.27(TF)+0.26(CMC)$	0.48
Coniferous	$Y=-19.01+0.20(TF)+0.30(MC)+3.94(OC)+0.06(TB)$	0.65

Y is propagule numbers of total fungi, CMC= soil CMCase activity, MC=moisture content, OC= organic carbon content, TB=numbers of total bacteria

the strong significance ($P<0.001$) of moisture content was observed in coniferous site where the moisture content ranged 17.6~25.1% of the dry weight throught a year.

On the other hand, the propagule numbers of *Trichoderma* spp. in deciduous and coniferous site showed commonly high ($P<0.01$) or strong ($P<0.001$) correlations with biotic parameters such as soil respiration ($r=0.744^{***}$, $r=0.726^{***}$, respectively), soil CMCase activity ($r=0.497^{**}$, $r=0.648^{***}$), number of total bacteria ($r=0.711^{***}$, $r=0.653^{***}$), and number of total fungi ($r=0.858^{***}$, $r=0.834^{***}$).

These results seemed to indicate the general trends that variations in propagule numbers of *Trichoderma* spp. were principally mediated by the actions of biotic factors rather than by the direct effects of abiotic environmental factors.

With a view to determine the variables most affecting distribution of *Trichoderma* spp., multiple-regression analyses were also carried out. In primary multiple-regression analysis, most of the independent variables were insignificant, and were hence omitted. The regressions were then recalculated with independent variables which showed significances ($P<0.05$) in first analysis. An shown in Table 5, statistically significant parameters for the distribution of *Trichoderma* spp. in deciduous site were number of total fungi and soil CMCase activity, and gave an R^2 value of 0.48. In equation for the distribution of *Trichoderma* spp. in coniferous site, number of total fungi, moisture content, number of total bacteria, and organic carbon content were the significant ($P<0.05$) independent variables and an R^2 values of this equation was 0.65. These resulting equations indicate that 48% and 65% of the variations in *Trichoderma* spp. propagules in deciduous and coniferous site, respectively could be accounted for by those environmental factors. As compared the regression coefficients with the simple linear correlation coefficients, same tendency in the action of factors to *Trichoderma* spp. was found. Because all possible influencing environmental factors were not measured in this study, it is difficult to provide satisfactory explanations for the relationships between *Trichoderma* spp. and the environmental factors on fungal propagules but the results of multiple-regression analyses are also indicating that biotic factors exert greater influences upon the variation in the occurrence of *Trichoderma* spp. than abiotic factors.

적 요

토양생태계내에서의 *Trichoderma* spp.의 생태학적 중요성을 이해하기 위하여 계룡산의 낙엽활엽수림과 침엽수림토양에서의 *Trichoderma*속에 속하는 종들의 계절과 土深에 따른 분포와 토양요인이 이들 균량의 변동에 미치는 영향에 대하여 조사하였다.

1. *Trichoderma* spp.의 총개체수는 하계에서 가장 높게 나타났으며 동계로 이행됨에 따라 점차 감소되었다. 전체 토양균류에 대한 *Trichoderma* spp.의 출현빈도율은 연평균 1.4% 이상으로 나타났다.

2. *Trichoderma* spp. 의 수직분포는 토심이 깊어질수록 총개체수와 전체균류에 대한 빈도율의 감소를 보였으며 토심에 따른 환경요인의 변화가 계절에 의한 환경요인의 변화보다 *Trichoderma* spp.의 균량변동에 크게 작용하였다.

3. 분리된 *Trichoderma* spp.의 동정결과 두 토양에서 공통적으로 *T. viride*, *T. polysporum*, *T. koningii*, *T. hamatum* 등의 순으로 출현빈도율이 높게 나타났으며 이들은 계절변동에 따라 각기 다른 출현양상을 보였다. *T. viride*는 춘계에서 추계에 걸쳐 우점종으로 나타난 반면, *T. polysporum*은 동계에서 우점종으로 나타났다.

4. 토양내 *Trichoderma* spp. 균량의 변동은 비생물적 환경요인의 직접적인 작용보다는 생물적 환경요인에 의해 지배받는 것으로 나타났으며 다중회귀 분석결과 활엽수림토양에서는 48%의 균량변동이 토양내 균류의 총개체수 및 토양내 섬유소분해효소의 활성도에 의해 지배받으며, 토양내 균류 및 세균의 총개체수, 수분함량 및 유기물함량이 침엽수림토양내의 *Trichoderma* spp. 균량변동의 65%를 지배하는 것으로 나타났다.

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