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Spore Germination of Some Fungi under Different Soil Conditions in Relation to Fungistasis

Min Woong Lee, Hyun Sung Shin* and Hae Jung Choi
Department of Agrobiology, Dongguk University, Seoul 110, and Department of Clinical Pathology Daejeon Medical Junior College, Daejeon 300-31, Korea*

土壤 條件에 따른 數種 菌類의 胞子 發芽와 靜菌現象

李 敏 雄・申 鉉 成*・崔 惠 貞
東國大學校 農生物學科・大田保健專門大學 臨床病理學科*

Abstract: Numbers of total bacteria and fungal propagules were highly populated in the soil of diseased plot in Gumsan, whereas numbers of actinomycetes were high in the soil of healthy plot in Goesan. In microbial fluctuation of different soil conditions four weeks after amended with various plant materials, bacteria were decreased in the soil amended with various plant materials as compared with non-amended soil except for Goesan. On the contrary, numbers of fungal propagules were increased amended with onion and potato stalk in two soils at different conditions, and also the propagules were enhanced in the soil amended with various plant materials in general. Higher numbers of bacteria and fungal propagules were observed in the diseased replanted plot in general, but germination rate of fungal spores relatively reversed against the microbial population. *Alternaria panax* was not affected much in their germination by natural soil condition, while *Fusarium* spp. were affected in their germination by natural soil. Natural soil showed a fungistatic effect against four fungal spores.

Keywords: Spore germination, Fungistasis, *Alternaria panax*, *Fusarium oxysporum*, *Fusarium solani*, *Fusarium moniliforme*, *Actinomycetes.*

An increase in soil microbial populations following organic and inorganic amendments is well known (Dutta and Isaac, 1979). The factors of fungal propagules to germinate in soil have been attributed to sufficient endogenous nutrients reserves and nutritionally deprived soil environment which is maintained by microbial competition (Ko and Lockwood, 1967; Yoder and Lockwood, 1973). Several workers proved that certain crop sequences, soil amendments and changs of C/N ratio decreased the incidence of *Verticillium* wilt disease in susceptible crops, and soil amendments rich in nitrogen were known to be effective to bring substantial reduction in the inoculum potentials of pathogens in soil (Papavizas, 1968; Powelson and Patil, 1963; Waksman, 1922; Whilhelm, 1951). Although many attempts have been made to suppress soil-borne plant pathogens by amendments (Garrett, 1965), very few studies have been reported on organic and inorganic materials in controlling some plant pathogens (Snyder *et al.*, 1959; Maier, 1961). The present study was undertaken to evaluate effectiveness of several organic materials on fluctuation of soil microorganisms and spore germination of four different fungal spores.

Materials and Methods

Characteristics of Soil

Soils were sieved (2 mm) before use, and the characteristics of these soils have been described by Son *et al.* (1985).

Maintenance of Propagules

Conidia from 5 days old culture of *Alternaria panax* Whetzel, *Fusarium oxysporum* Schlect, *F. solani* (Mart.) Appel and Wr., and *F. moniliforme* Scheldon were maintained on potato dextrose agar. Culture transfers were routinely derived from one parent culture of each fungus.

Preparation of Propagules Suspension

Membrane filters bearing the conidia (Filonow and Lockwood, 1979) were prepared for the spore germination assays on natural soil, sterile soil and amended soil with various plant materials (Zakaria, 1978).

Microbial Populations in Soil

Microbial populations in soils were estimated by dilution plate counts on the following media; Chitin medium (Hsu and Lockwood, 1975) for actinomycetes, Martin's rose bengal medium (Martin, 1950) for fungal propagules, and Hutchinson medium (Bhat and Shetty, 1942) for total bacteria. Plates were streaked with 0.2 ml of soil suspension and incubated at 24°C 5-7 days for bacteria and fungi and 10 days for actinomycetes.

Sensitivity of Propagules to Soil Microorganisms

Germination of propagules was determined on sterilized soil, natural soil and amended soil with 1% ground plant materials (Zakaria, 1978). Fifty gram samples of the soil were wetted to 30% WHC, and equillibrated for 16~24 hr before use. The soils in 9 cm Petri dish were sterilized by autoclaving for 1 hr as a sterile soil. Natural soils and amended soils were contained in Petri dish. The soil was well mixed with spatula, smoothed and allowed to equilibrate for 1 hr. In each experiment, duplicate Nuclepore membranes (0.4 μm pore dia, Nuclepore Corp., Pleasanton, CA) bearing fungal propagules were placed on duplicate samples of sterile soil, natural soil and amended soil. Conidia were incubated on soil for 12-14 hr prior to germination assay. Membranes bearing propagules were stained with phenolic rose bengal solution (Hsu and Lockwood, 1973), destained in water, and mounted on glass slide with double sticky tape. Spores with germ tubes equal in length to the width of the spore considered as germination. Germination counts were made 200 spores on each of two membranes.

Results

Numbers of bacteria and fungal propagules were distributed in higher numbers in the soil of diseased plot at replanted ginseng field (diseased plot of soil) in Gumsan (Table I, II). On the contrary, the numbers of actinomycetes were highly populated in the soil of healthy plot at first planted ginseng field (healthy plot of soil) in Goesan (Table III). For the popula-

Table I. Numbers of bacteria in different soil conditions of two locations where ginseng was cultivated (5×10^5/g soil).

Location	No. of bacteria (5×10^5/g soil)	
	Healthy plot[a]	Diseased plot
Gumsan	24.6a[b]	153.3b
Goesan	6.7a	28.3a

a. Healthy plot: Healthy plot of soil in first planted field of ginseng.
Diseased plot: Diseased plot of soil in replanted field of ginseng.

b. The different letters in column indicate a significant difference (P=0.01) according to Duncan's new multiple range test.

Table II. Numbers of fungal propagules in different soil conditions of two locations where ginseng was cultivated (5×10^3/g soil).

Location	No. of fungal propagules (5×10^3/g soil)	
	Healthy plot[a]	Diseased plot
Gumsan	17.3a[b]	106.6b
Goesan	9.3a	3.0a

a. Healthy plot: Healthy plot of soil in first planted field of ginseng.
Diseased plot: Diseased plot of soil in replanted field of ginseng.
b. The different letters in column indicate a significant difference (P=0.01) according to Duncan's new multiple range test.

Table III. Populations of actinomycetes in different soil conditions of two locations where ginseng was cultivated ($\times10^6$/g soil).

Location	No. of actinomycetes ($\times10^6$/g soil)	
	Healthy plot[a]	Diseased plot
Gumsan	9.2a[b]	9.2a
Goesan	72.1b	13.6a

a. Healthy plot: Healthy plot of soil in first planted field of ginseng.
Diseased plot: Diseased plot of soil in replanted field of ginseng.
b. The different letters in column indicate a significant difference (p=0.01) according to Duncan's multiple range test.

Table IV. Population changes of bacteria in two soil conditions in Gumsan and Goesan 4 weeks after amendments with 1% (w/w) ground plant materials.

	No. of bacteria ($\times10^6$/g soil)			
	Gumsan		Goesan	
	Healthy plot	Diseased plot	Healthy plot	Diseased plot
Control	17.3a	195.3b	9.3a	3.0a
Onion stalk	10.0a	25.0a	175.7b	19.0a
Corn stalk	7.0a	27.3a	78.3a	24.0b
Potato stalk	21.3a	130.0a	635.3cd	5.7a
Garlic stalk	17.3a	21.3a	37.7a	5.0a

The different letter in column indicate a significant difference (P=0.01) according to Duncan's new multiple range test.

tion changes of bacteria 4 weeks after amendments with plant materials, the numbers were decreased in amended soil as compared to natural soil except healthy field of Goesan, but the numbers increased only in a treatment with corn stalk in diseased plot in Goesan, and also its numbers were increased in amendments with potato stalk, and onion stalk in Goesan healthy plot of soil (Table IV). In the diseased plot at Gumsan, the numbers were not significantly increased in the soil amended with various plant materials (P=0.01). For the fluctuation of fungal propagules, higher numbers were presented in the soil amended with corn stalk and potato stalk in the soil of two location in general, but the numbers were decreased in the soil treated with garlic stalk. Especially higher fungal propagules were present in the diseased plot amended with potato stalk in general. The fungal propagules were more generally increased with the treatment of plant materials than non-amended soil conditions (Table V).

In an investigation of germination rate in Gumsan soil after amended with various plant materials, germination rates of *A. panax* were 37% and 68.1% in natural soil in two soil conditions, but the rates were generally decreased in amended soil with plant materials except sterile soil. In case of *F. solani*, the germination rates

Table V. Population changes of fungal propagules in two soil conditions in Gumsan and Goesan 4 weeks after amendments with 1% (w/w) ground plant materials.

	No. of fungal propagules (5×10^3/g soil)			
	Gumsan		Goesan	
	Healthy plot	Diseased plot	Healthy plot	Diseased plot
Control	24.7a	153.0a	6.7a	28.3b
Onion Stalk	136.7b	260.0b	34.0b	49.3bc
Corn stalk	254.0bc	107.0a	22.3a	29.3b
Potato stalk	309.0bc	260.0b	23.7b	45.0bc
Garlic stalk	162.0b	129.0a	17.0a	9.0a

The different letter in column indicate a significant difference (P=0.01) according to Duncan's new multiple range test.

Table VI. Germination rate of *Alternaria panax*, *Fusarium solani*, *F. moniliforme* and *F. oxysporum* in two soil conditions in Gumsan 4 weeks after amendments with 1% (w/w) ground plant materials.

	Germination (%)							
	Healthy plot				Diseased plot			
	Ap	Fs	Fm	Fo	Ap	Fs	Fm	Fo
Control	68.1	11.2	27.7	12.0	37.0	5.0	24.6	10.4
Onion Stalk	10.7	4.8	17.0	13.1	25.4	3.0	13.5	3.3
Corn stalk	16.7	9.3	27.1	47.6	26.1	1.9	10.0	5.3
Potato stalk	19.9	11.9	23.1	15.0	26.4	3.0	14.4	8.7
Garlic stalk	24.8	9.1	33.3	19.6	14.6	4.5	22.4	1.2
Sterile soil	94.0	90.0	93.0	92.5	93.0	90.0	92.8	92.5

Ap: *A. panax*, Fs: *F. solani*, Fm: *F. moniliforme*, Fo: *F. oxysporum*

were surely low in the diseased plot and healthy plot of soil. The rates of *F. moniliforme* were relatively lower in its germination amended with plant materials than non-amended natural soils. The germination rates were low in the soil conditions amended with onion stalk in healthy plot of Gumsan, and corn and onion stalk in diseased plot of Gumsan. The rates of *F. oxysporum* were low in the soil amended with garlic and onion stalk in diseased plot and the soil amended by corn stalk increased the germination rate in healthy plot than control (Table VI). The treatment with onion stalk decreased the germination rate in the healthy plot of soil and diseased plot of soil in general.

In soil samples of Goesan, the germination rates of *A. panax* were low in the soil amended with potato stalk and corn stalk in the healthy plot of soil, but the germination rates were increased in onion stalk. The rates were low in corn stalk treatment in diseased plot of soil (Table VII). For the germination of *F. moniliforme* and *F. oxysporum*, onion stalk and corn stalk lowered the rate in healthy soil. The germination rates of *F. solani* were the lowest among 4 fungal spores in two plots of soil (Table VI, VII). There was difference among the fungal propagules in their germination responses to soil conditions; germination rate was high in healthy field of soil as compared to diseased plot of soil. However

Table VII. Germination rate of *Alternaria panax*, *Fusarium solani*, *F. moniliforme* and *F. oxysporum* in two soil conditions in Goesan 4 weeks after amendments with 1% (w/w) ground plant materials.

	Germination (%)							
	Healthy plot				Diseased plot			
	Ap	Fs	Fm	Fo	Ap	Fs	Fm	Fo
Control	39.8	2.5	13.8	8.0	46.8	9.0	22.2	27.1
Onion stalk	47.4	4.4	7.9	4.2	16.5	8.2	15.5	25.1
Corn stalk	26.8	3.1	7.0	6.7	11.2	6.2	11.6	18.3
Potato stalk	10.0	3.5	19.9	20.6	39.9	22.9	30.8	29.6
Garlic stalk	34.7	2.0	8.2	7.0	22.9	17.1	23.1	16.6
Sterile soil	94.5	89.0	90.0	90.0	94.0	90.0	93.0	92.5

Ap: *A. panax*, Fs: *F. solani*, Fm: *F. moniliforme*, Fo: *F. oxysporum*.

there was a few exception in their germination rate(Table VI, VII). The four fungi germinated from 89-94 percent on autoclaved sterile soil.

Discussion

It is established fact that in agricultural soils higher level of available nitrogen hasten the decomposition of plant residue accompanied by a rapid increase in the populations of competitive soil microorganisms (Baker and Cook, 1974; Smith and Douglas, 1971). In this study numbers of fungal propagules of natural soil of diseased plot in Gumsan and Goesan were higher than healthy plot of soil, but the germination ratios were reverse against the microbial numbers in soil (Table I, II). In a diseased field soil of Gumsan, amendments with onion stalk and potato stalk increased the population of of fungal propagules, but declined the germination rate of the four fungal spores as compared to non-amended natural soil. The population density amended with garlic stalk did not increase the population of bacteria and fungal propagules in two soil conditions, and also it lowered the germination rate four fungal spores. In healthy field of Gumsan also fungal propagules were increased in the treatment with potato stalk, corn stalk and onion stalk, but on the contrary bacterial numbers were decreased in the amended soil conditions as compared to non-amended natural soil (Table IV, V). Henis *et al.* (1967) observed that the effect of amending soil with plant residue was to reduce the inoculum density of certain plant pathogens through changes in the general microbial balance of soil (Baker and Cook, 1974; Garrett, 1970; 1975), and Graffar *et al.* (1969) also proved that soil amended with either alfalfa or barley straw increased the population of bacteria and actinomycetes. *A. panax* among four fungal spores especially germinated high in all soil conditions. It seemed that large and rapidly germinating spores showed a low fungistatic sensitivities, since rapid germination would tend to complete the germination process before depletion of substrate by microbial competition (Steiner and Lockwood, 1969). In this study the results showed higher numbers of bacteria and fungal propagules observed in diseased plot of soil in general, but germination rates of four fungal spores relatively reverse against the microbial population. The soils had a higher numbers of microbial populations the lower rates of germination are observed in those soils. Soil amended with various plant materials usually had increased fungal propagules, but bacterial cells were exceptional in healthy soils of Goesan (Adams *et al.*, 1918; Lee and Choi, 1982). Most fungi survive in the form of resting spores, hyphae, or sclerotia which are intimately associated with phenomenon of soil fungistasis in natural soil. The importance of exogenous nutrients on germination of spores has been also discussed by Lockwood and coworker (Lockwood, 1975; Steiner and Lockwood, 1970). The fungistatic conditions in soil may or may not be annulled by appropriate nutrients (Hora and Baker, 1970; 1975; Romine and Baker, 1973). In this experiment fungistasis was largely annulled autoclave soil, but natural soil showed a fungistatic effect to four fungal spores germination. The lower rate of germination of fungi may be attributed to the higher numbers of microorganisms as to greater microbial activity. It also lead to a higher fungistatic condition in soil.

摘 要

全 細菌과 菌類는 錦山의 再作 罹病圃 土壤에 많이 分布하였고 反面 放線菌의 數는 槐山의 初作健蔘圃 土壤에 많이 分布하였다. 材料 土壤을 여러가지 植物質로 改良處理 후에 土壤中의 微生物 數의 變動을 조사한 결과, 槐山 地域의 土壤을 제외하고 다른 土壤에서는 細菌의 數가 減少하였고, 반대로 菌數는 2個 地域 土壤에서 양파와 감자줄기를 처리한 土壤條件에 數가 增加하였다. 또한 改良 處理한 土壤에서 일반적으로 菌類가 증가하였다. 細菌과 菌數는 罹病 再作地에서 일반적으로 增加하였다. 菌의 胞子 發芽는 微生物의 分布數가 많을수록 낮아졌다. *A. panax*는 自然土壤條件에서 胞子 發芽가 적게 영향을 받았으나, *Fusarium*屬은 영향을 받았다. 自然 土壤은 4가지 菌의 胞子 發芽力에 대해 靜菌現象을 나타내었다.

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