Effects of Fungicidal Drenches on Damping-off Organisms in Ginseng Seed Bed and Yield of the Seedling Root*

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살균제의 토양관주에 따른 인삼모잘룩병균(子苗立枯病菌)의 숫적변화(數的變化) 및 묘삼뿌리의 수량

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Abstract

1. The number of *Rhizoctonia solani*, *Pythium debaryanum*, *Fusarium* and *Trichoderma* was detected by the modification of Boosails special plating method when ginseng seed bed was drenched with Captan, Difolatan, Zineb, Maneb and PCNB at weekly intervals. *Pythium debaryanum* Hesse was described for the first time on *Panax ginseng* in Korea.
2. The number of *Rhizoctonia solani* and *Pythium debaryanum* was decreased gradually as the season progressed, whereas that of *Fusarium* and *Trichoderma* was increased.
3. The number of *Rhizoctonia solani* was greatly reduced by PCNB, and soils treated with other fungicides generally showed less *Rhizoctonia solani* than in the control. The number of *Pythium debaryanum* was significantly reduced by Zineb, Maneb, followed by Captan and Difolatan. None of the fungicids reduced the number of *Fusarium* colonies in the fourth week. Effects of the chemicals on *Trichoderma* were not statistically significant.
4. More fresh weight of the seedling roots was obtained using Difolatan, Maneb and PCNB. Phytotoxicity was noted with Maneb, Zineb and Captan after the third treatment.

Introduction

Ginseng is at present grown in 33 districts in Korea under a government monopoly system and ginseng exports have shown a rapid increase, year after year from 1.6 million dollars in 1968.

Because the seedlings grow close together in dense

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stands and the plants remain in the field for several years, severe epidemics of soil borne diseases as well as diseases of aerial parts of the plants often occur. Among the diseases on ginseng, severe damping-off in seed beds is one of the most important diseases. The losses due to damping-off are about 10 to 30 percent throughout Korea [8] (19% at Puyo, 30% at Kumsan [9]), about 50% in the United States [19]. Therefore the farmers are faced with a shortage of seedlings to be transplanted every year.

Extensive studies have been made on damping-off of seedlings and its control [5,16,17,20]. Vaartaja [16] screened 109 chemicals against *Rhizoctonia solani* and *Pythium debaryanum* with pine and birch seeds in laboratory tests. Among them Rimocidin, Captan and Thiram at a wide range of concentration, controlled the diseases without marked phytotoxicity and 19 chemicals performed well at narrow concentration ranges. Cram et al. [3] tested Thiram and Captan on unsterilized soil infested with *R. solani*, counting the number of damping-off pine seedlings. They obtained good control against the inoculum fungus and natural *Pythium* flora by the two chemicals. PCNB has been well known as one of the excellent chemicals against *R. solani*[4,6,7,18]. Gibson [7] described an anomalous effect of PCNB on *P. debaryanum*. PCNB not only failed to control the disease caused by the fungus but its use was followed by appreciably increased disease development.

The early literature which deals with the diseases of ginseng was reviewed by Nakata in 1922 [11]. Recently, the diseases of ginseng received little attention not only in Korea but also throughout the world. Wetzel [19] and Nakada [11] described symptoms, causal organisms and control of some important ginseng diseases including damping-off in the United States and in Korea, respectively. On *Panax quinquefolium*, *R. solani* and *P. debaryanum* were described as damping-off organisms [15,19]. Whereas on *Panax ginseng* only *R. solani* was described [12]. Bunkina [2] identified 25 fungi from ginseng, among them *Fusarium* spp. and *R. solani* occurred on seedlings and roots obtained from plants of Primor'e and Korea origin.

In Korea, Hong [9] reported the percentage of losses due to diseases in ginseng growing regions. Lee et al. [10] reported number of fungi and bacteria from ginseng growing soils in addition to observations of several soil borne diseases.

The purpose of the present study was to investigate the effects of fungicidal drenches on damping-off organisms in ginseng seed bed and yield of the seedling root.

Table 1. Active ingredients and dilutions of fungicides used in soil drenching.

Fungicide	Active ingredient	Dilution or amount
Captan 50%	(N-trichloromethylthio-1,2,3,6-tetrahydrophthalimide)	1 : 600
Zineb 75%	(Zinc ethylene-bis-thiocarbamate)	1 : 600
Maneb 70%	(Manganous ethylene-bis-dithiocarbamate)	1 : 800
Difolatan 80%	(N-tetrachloroethylthio 4-cyclohexene-1,2-dicarboximide)	1 : 1,000
PCNB 20%	(Pentachloronitrobenzene)	1kg/a

Materials and Methods

Soil drenches

The experiment was carried out in 1968 on a seed bed cultivated by a farmer at Yongin, Korea. The soil has not been treated with fungicides prior to the experiment.

The chemicals (Table 1) were applied as surface drenches to soil 5 times at weekly intervals, on June 1, 8, 15, 22, and 29. With each chemical a suspension of 900 ml was applied to each plot, the plots being randomized with 3 replications. The plot size was 40 cm^2, large enough for about 80-100 seedlings. The experimental field was 16.8m long and 0.78m wide. Soil samples of 150-200g were taken at a depth of 5-10 cm from each plot before treatment and after the

second and the fourth treatments. The roots of the ginseng seedlings were harvested on November 3, 1968.

Screening of organisms

The special plating methods of Boosalis (1) were modified to isolate and determine damping-off organisms. One hundred g. each of the eighteen sample of soil was taken. A random sample of 100 plant debris particles from each sample of soil was transferred to 25 perti plates containing 2 percent water agar. The pH of the medium was 5.6~6.0. The streptomycin sulfate was used for prevention of contamination of bacteria. The plate was incubated for 3 days at 25±1°C.

Colonies of *Fusarium*, *Trichoderma*, *R. solani*, and *P. debaryanum* were detected by their characteristics on the medium. *Mucor* and some unidentified genera were not counted.

Results

Detection of organisms by plating

The colonies of *R. solani*, *P. debaryanum*, *Fusarium* and *Trichoderma* were detected in water agar. The mycelial growth of the fungi in water agar was observed under diffused light from window with naked eye. A microscope was used for detection of uncertain fungi.

R. solani could be distinguished from the other fungi with mycelial growth within 48 hours. The hyphae branched irregularly and their subdivisions occurred typically at right angles. One of the typical characteristics of *R. solani* was that the colonies had branch shaped mycelial threads and broader margins than those of the other fungi.

The colonies of *P. debaryanum* were white with flat mycelial growth. The mycelial threads were more or less creeping on the agar surface.

The branched hyphae were more subdivided than those of *R. solani*, *Fusarium* and *Trichoderma*.

The margins of *Fusarium* and *Trichoderma* were circular. The clusters of conidia could be observed on mycelial threads in *Fusarium*. The hyphae of *Fusarium* were branched to radial symmetry. The mycelia of *Trichoderma* were more branched than those of *Fusarium* and the cushion of conidia was green in colonies of *Trichoderma*.

Effects of fungicidal drenches on population dynamics of ginseng damping-off organisms

Frequency of plant debris infested with *R. solani* was the lowest, with a minimum of one and a maximum of nine (Table 2). PCNB greatly reduced the number of *R. solani* gradually during the period of soil drenching. Similar trends were observed with Difolatan and the other fungicides. The fungicide-treated soils generally showed less *R. solani* than in the control.

The colonies of *P. debaryanum* were detected with higher frequencies than the other organisms, with as many as 58 colonies per 100 plant debris particles in the control. As a whole, a gradual decrease in number of colonies was observed with increasing soil drenches. Zineb was the most effective in reducing the number of *P. debrayanum*.

The number of *Fusarium* colonies was quite variable among the plots even before the soil drenches. None of the fungicides reduced the number of *Fusarium* colonies in the fourth week. With some of the fungicides there was a decrease in number of colonies after the second treatment, followed by a later increase.

The number of *Trichoderma* was also quite variable. The numbers of this fungus were gradually reduced by Maneb and Zineb. The number increased at the second treatment with PCNB and Difolatan and then decreased at the fourth week.

Differences in numbers of organisms with different treatments were statistically significant at 1 percent level except in *Trichoderma*. No significant differences were found in numbers of organisms for blocks and chemical treatments with exception of *Pythium debaryanum* ($p=0.95$). Differences in numbers of *Trichoderma* were nonsignificant.

Table 2. Effects of fungicidal drenches on population dynamics of ginseng damping-off organisms and *Trichoderma* in soil

Treatment[t]	Number of colonies per 100g soil yielding after drenches[a)]											
	R. solani [b)]			*P. debrayanum*			*Fusarium*			*Trichoderma*		
	0	2nd	4th	0	2nd	4th	0	2nd	4th	0	2nd	4th
Control	9	8	5	58	31	24	13	16	23	26	32	37
Captan	6	4	2	46	17	18	33	14	42	26	25	32
Difolatan	9	5	3	51	21	23	16	36	36	17	24	12
Maneb	6	5	2	56	19	13	23	15	36	37	23	18
Zineb	7	4	3	50	19	10	13	8	20	29	25	24
PCNB	9	4	1	55	48	36	19	22	46	27	37	27

a): Averages of 3 replications per 100g soil samples
b): Drenches at weekly intervals

Table 3. Effects of fungicidal drenches on fresh weight of ginseng seedling roots[a)]

Treatment	Fresh weight of seedling roots[b)] (g)
Control	39.6 yz
Captan	22.0 y
Difolatan	48.6 z
Maneb	49.3 z
PCNB	49.3 z
Zineb	30.0 y

a): 15 month old roots harvested on November 3, 1968.
b): Averages of g/40cm², 3 replications
Numbers followed by the same letter are not significantly different at 5 percent level.

Effects of fungicidal drenches on ginseng seedling growth

Captan, Maneb and Zineb resulted in phytotoxic symptoms in the ginseng seedlings after the third treatment. Brownish spots and bleaching from edge of the leaves were observed on the oldest leaves. The phytotoxic symptoms appeared in May and gradually more increased from July to August. No remarkable phytotoxicity was found in the plots treated with PCNB and Difolatan. More fresh weight of seedling roots was obtained using Difolatan, Maneb and PCNB than in the control. On the contrary, with Captan and Zineb the weight was less than in the control. Differences in the weight between the two groups of chemicals were statistically significant at 5 percent level(Table 3).

DISCUSSION

The effective screening of damping-off pathogens from soil has been studied with various techniques; however, no simple method is known to be satisfactory for screening of all damping-off pathogens. It was agreed with Boosalis' [1] description that *Rhizoctonia* could not be isolated by the commom dilution method. In preliminary experiment, neither tissue transplants or dilution method was desirable for the isolation of *Pythium*. The authors considered that modification of the special plating method using water agar described by Boosalis [1] was desirable to isolate not only *R. solani* but also *P. debaryanum*, *Fusarium* and *Trichoderma*.

P. debaryanum Hesse was first described by the authors as a damping-off organism on *Panax ginseng* in Korea. The same fungus was described on *Panax quinquefolium* in the United States[15,19].

It has been well known that *P. debaryanum* associated with small fragments of dead plant tissues in soil is a frequent source of potential inoculum for damping-off of seedlings. Probably difficulties of technique resulted in failure to isolate the organism for *P. gineng*. In the present study, *P. debaryanum* could be screened by special plating method using water agar with the most abundant colonies of the fungus assoc

iated with plant residues.

The phenomenon that fungicides are highly specific in their action has been well known [4, 6, 7, 14]. In the present study, PCNB was effective to reduce the number of *R. solani*, whereas the number of *P. debaryanum* was greater with PCNB than in the control. The result agrees with Gibson's report [7] that damping-off caused by *Pythium* sp. was decreased. Maneb and Zineb caused a decreas in the number of *P. debaryanum*. The number of *Fusarium* was not reduced by any fungicide tested in this study. In *Trichoderma* the number of colonies varied irregularly. In the control the number of *R. solani* and *P. debaryanum* decreased in process of time. This coincided with a decrease of damping-off in ginseng seedlings. Probably the decrease of population of the pathogens in soil led to the decrease of damping-off in ginseng seedlings. Fulton[6] reported that *R. solani* was considerably more active at lower temperatures than *Sclerotium bataticola* and *Rhizopus* sp.

Trichoderma lignorum is known to be an antagonistic microorganism against *R. solani*[18]. Gregory[8] reported that application of *T. lignorum* into sterile soil infested with *P. debaryanum* completely prevented damping-off of alfalfa. In the present study the number of colonies of *Trichoderma* varied with the fungicides. For instance, Maneb resulted in reduction whereas with PCNB the number increased. Fungicidal effect against antagonistic organisms such as *Trichoderma* was found abundantly in ginseng seed beds, application of *Trichoderma* for control of ginseng damping-off would be a subject for future research.

적 요

1. 인삼묘포에 Captan, Difolatan, Zineb, Maneb와 PCNB를 일주일 간격으로 토양관주 하였다. 처리하지전과 2회 및 4회처리후에 각각 *Rhizoctonia solani*, *Pythium debaryanum*, *Fusarium* 그리고 *Trichoderma* 균총의 숫적(數的)변화를 Boosalis의 특별잔사법에 의해서 조사하였다. 우리나라에서 인삼(*Panax ginseng*)의 모잘록병균으로서 *Pythium debaryanum*을 처음으로 기술하였다.
2. *Rhizoctonia solani*, *Pythium debaryanum*의 수는 토양관주에 관계없이 시일이 경과함에 따라서 점차로 감소하였고 *Fusarium* 및 *Trichoderma*는 오히려 증가하였다. *Rhizoctonia solani*의 수는 PCNB에 의해서 현저하게 감소하였고 다른 약제에 의해서도 대조구 보다 감소하였다.
3. *Pythium debaryanum*은 Zineb, Maneb, Captan, Difolatan의 순으로 그 수가 증가하는 반면 *Fusarium*은 일정한 경향을 볼 수 없었다. *Tichroderma*를 제외한 모든 균의 숫적 변화는 시일의 경과에 대하여 1% 수준에서 유의성이 있었다.
4. 토양관주에 의한 약해는 Maneb, Zineb, Captan구에서 나타났고 묘삼 뿌리의 생체 무게는 Difolatan, PCNB, Maneb구에서 대조구 보다 증가하였다.

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<초 록> 벼도열병방제를 위한 강우시의 약제산포효과

식물환경연구소. 병리담당관실. 이 경 휘·정 봉 구·김 광 석

벼의 병해중 그 피해가 심한 도열병의 방제 적기에 빈번한 강우로 약제 산포를 못하고 있는 실정이므로 1970년 7월에 그 실제 효과를 분석 검토 하고저 온실내에서 인공강우로서 방제효과를 조사하였다.

공시 품종은 팔달이고 육묘대로 3~4 엽기까지 재배하였으며 공시약제는 유제로서 부라에스와 히노산이 각각 1 : 1000 이었고 분제로서 히노산과 가스가민분제를 4kg/10a 기준으로 산포하였다. 병원균의 접종은 약제산포 1 일전에 분생포자 현탁액을 (포자농도 10~15개) 유묘에 분무접종하였고 인공 강우는 70cm 높이에서 30 초간조로 물로 세정한 것으로 하였다. 그리고 강우 처리시간은 1) 약제산포후 무강우 2) 약제산포 2시간후 강우 3) 약제산포 4시간후 강우 4) 약제산포 6시간후 강우 5) 약제산포 24 시간후 강우 6) 약제산포 48 시간후 강우 7) 병원균만의 접종구 등으로 나누었다.

표 1.에서와 같이 접종구에 대한 백분율로 비교하여 보면

Table 1. Effect of the application of agricultural chemicals for the control of blast fungus in artificial rainfall.

Hour of rainfall Agri-chemicals used	Rainfall after 2-hours	After 4 hrs	After 6 hrs	After 24hrs	After 48hrs	Check
Bla's E.C.	47	58	71	77	80	85
Hinosan E.C.	40	42	57	76	84	85
Kasukamin D.	71	83	77	79	88	96
Hinosan D.	55	69	76	89	91	96

※ Illustrated numbers show control percentage

약제산포후 2시간, 4시간, 6시간, 24시간, 48시간후 강우는 약제의 종류에 따라 방제효과는 다르나 첫째 약제산포 2시간후 강우의 경우에도 최소한 히노산유제산포구가 40%, 부라에스 유제구가 47%, 히노산 분제구가 55% 끝으로 가스가민 분제구가 71%의 방제효과가 있었다. 결론적으로 4시간 이후의 강우는 거의 방제지장이 없음을 알수 있었다.