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Relationship between Entomopathogenic Nematode and Entomopathogenic Fungus, *Beauveria brongniartii*

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곤충병원성 선충과 곤충병원성 곰팡이, *Beauveria brongniartii*와의 상호관계

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ABSTRACT

Interrelationship of entomopathogenic nematodes within nematode species and between entomopathogenic fungus, *Beauveria brongniartii* was investigated to enhance nematode efficacy. The matured adults of *S. glaseri* were not different depending on host weight but different in infective juveniles propagated. When several concentrations of infective juveniles were inoculated to host at 20℃ or 30℃, the number of females and males matured in the host was variable both in *S. glaseri* and in *S. monticola* depending on concentrations. Total matured adults at the concentrations of over 40 infective juveniles were more than at those of below 20 ones. Although there showed no difference in infective juveniles propagated depending on concentrations, infective juveniles were significantly low at high concentration, 1,000 infective juveniles in both species and temperature. Steinernematids generally outcompeted heterorhabditids when they were inoculated together to the same host in a container. Mortalities were 76.2±4.8 by *S. carpocapsae* Pocheon and 23.8 ±4.8 by *H. bacteriophora* Hamyang. When *S. carpocapsae* Pocheon and *H. bacteriophora* NC 1 were inoculated together, mortalities were 90.5±4.8 by *S. carpocapsae* Pocheon and 9.5±4.8 by *H. bacteriophora* NC 1. The similar trends were shown in the combination of

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S. glaseri NC (61.9±9.5% and 80.9±4.8%) and *H. bacteriophora* Hamyang (38.1±9.5%) or *H. bacteriophora* NC 1 (19.1±4.8%). When entomopathogenic nematodes were inoculated with *B. brongniartii* to a host, co-infection with fungus was observed from 12 hr pre-treatment of *B. brongniartii* with *S. carpocapsae* All and 6 hr pre-treatment of *B. brongniartii* with *H. bacteriophora* NC 1. Nematode, fungus, and nematode-fungus co-infection were observed from 48 hr pre-treatment of *B. brongniartii*. However, infective juveniles emerged from co-infected cadavers were much fewer than nematode alone infection.

Key words: interrelationship, infective juvenile, *Steinernema carpocapsae*, *S. monticola*, *S. glaseri*, *Heterorhabditis bacteriophora*, *Beauveria brongniartii*

INTRODUCTION

Entomopathogenic nematodes in the families *Steinernematidae* and *Heterorhabditidae* are obligate pathogens in nature and kill their hosts with the assistance of specific symbiotically associated bacteria (*Xenorhabdus* spp. for steinernematids and *Photorhabdus luminescens* for heterorhabditids) (Kaya and Koppenhöfer, 1996). Thus these nematodes have received considerable attention as bioinsecticides because of having a broad host range with highly virulence against a number of insect pests, killing their hosts rapidly, applying easily with standard spray equipment, reproducing massively *in vivo* or *in vitro*, being capable of using for suppression of target pests in environmentally sensitive areas and being compatible with other control agents. These nematodes are especially effective to insects in soil and cryptic habitats and much efforts have also been made to control insect pests in other habitats. The strategies for utilization of entomopathogenic nematodes have developed to obtain satisfactory results. Combination with other control methods such as chemicals and entomopathogens including different species of nematodes are the improvements to reinforce nematode efficacy. Intrarelationship or interrelationship of nematodes between nematodes themselves and other pathogens need to be studied for efficient control. Moreover, host size may influence progeny production of nematodes *in vivo* owing to intrarelationship of nematodes. Nematode efficacy may be also dependent on size or weight of target pests by the same mechanism. In fact, combination application of two species of entomopathogenic nematodes with different search strategies or with other entomopathogens have been recently developed to enhance nematode efficacy against the same host or different hosts sharing the same habitat (Kaya, 1993; Kaya *et al.*, 1995; Thurston *et al.*, 1993; Choo *et al.*, 1996, 1997; Koppenhöfer and Kaya, 1997; Koppenhöfer *et al.*, 1999). Thus, our study was conducted to investigate relationship of nematodes with different species or with other micropathogens in order to reinforce nematode efficacy and to see intrarelationship within a host.

MATERIALS AND METHODS

Nematode. Four species were need; *Steinernema carpocapsae* All and Pocheon, *S. glaseri* NC, *S. monticola* and *Heterorhabditis bacteriophora* Hamyang and NC 1. The nematodes were produced in the last instar of *Galleria mellonella* larvae and juveniles were harvested using white traps (Woodring and Kaya, 1988). Nematode suspension was maintained at 10℃ at a concentration of 1,000 nematodes/mℓ and diluted to the desired concentration as needed. Nematodes were used within 3 weeks after harvest.

Intrarelationship in the same host. *G. mellonella* larvae were weighed and divided into two groups, <120mg (68~118mg) and >350mg (351~407mg). One larva was placed into 60×15mm Petri dish containing filter paper moistened with 0.5mℓ sterilized water and 15 juveniles of *S. glaseri* NC were inoculated with mouth pipette. Petri dishes were grouped for checking matured adults and progeny production. The cadavers were dissected 3 days after inoculation and females and males were counted from each cadaver. Another group was trapped using 60×15mm petri dish and juveniles emerged were counted everyday. In addition, the sand was sieved with 850μm sieve and sterilized twice at 121℃, 15psi for 30 minutes. *G. mellonella* larva (180±10mg) was placed at bottom of 30mℓ plastic cup and filled with 25mℓ of sand (10% moisture). Every cup received *S. glaseri* NC or *S. monticola* at the concentrations of 0, 10, 20, 40, 60, 100, 150, 200, 250, 500, or 1,000 infective juveniles/0.5mℓ. The cups were kept at either 20℃ or 30℃ incubators. The cadavers were removed and dissected 3 days after inoculation. Then, females and males of nematodes were counted from each cadaver. Cadavers were also trapped and kept at 20℃ or 30℃ incubators to harvest progenies. The infective juveniles emerged from trapped cadavers were counted everyday. The test was made three replicates with 15 cups per replicate.

Interaction between nematode species and entomopathogenic fungus. The sand was prepared described as above. A *G. mellonella* larva (170±30mg) was placed into 30mℓ plastic cup and filled with 25mℓ of sand (10% moisture). The treatments were grouped into *S. carpocapsae* Pocheon (ScP) only, *S. glaseri* NC (SgNC) only, *H. bacteriophora* NC 1 (HbNC1) only, *H. bacteriophora* Hamyang (HbH) only, ScP+HbN, ScP+HbH, SgNC+HbNC1, and SgNC+HbH. 30 infective juveniles/0.5mℓ were inoculated into one nematode species group and 15 infective juveniles of each nematode species were inoculated into combination group. The cups were kept at 20℃ or 30℃ incubators and the others were processed described as above. The test was made three replicates with 7 cups per replicate. On the other hand, 10 *Galleria* larvae were placed into 90×15mm petri dishes and entomopathogenic fungus, *Beauveria brongniartii* (Bb) produced on SMAY medium was inoculated at a concentration of 4.8×10^5/㎖. 200 *S. carpocapsae* All and 150 *H. bacteriophora* NC 1 were treated at 0, 6, 12, 24, 48, 72, and 96 hr after fungus inoculation. Then, petri dishes were wrapped with 0.02㎜ of plastic bag and kept at 25±2℃ incubator. The cadavers were checked which pathogens killed hosts and nematode progenies emerged from cadavers were counted. The test was made as 5 replicates with 12 petri dishes per replicate.

Statistical analysis. Analysis of variance (ANOVA) was used to determine the significant differences. Means were used to compare differences among treatments using Duncan's multiple-range test (DMRT), χ^2-test and Student's *t*-test (SAS Institute, 1988).

RESULTS

Intrarelationship in the same host. The matured adults or sex ratios of *S. glaseri* NC were not different depending on host weight (χ^2=1.3; df=1; P=0.3) (Fig. 1). In group of <120㎎ the number of females per host was 2.2±1.8 and males 2.4±2.0. And in group of >350㎎ that of females was 2.6±0.4 and males was 1.6±0.3. However, infective juveniles emerged were significantly different according to host weight (Fig. 2). Total number was more in >350㎎ than in <120㎎ but number per ㎎ was more in <120㎎ than in >350㎎. Total number of infective juveniles emerged per host was 36383.6±7825.0 in <120㎎ and

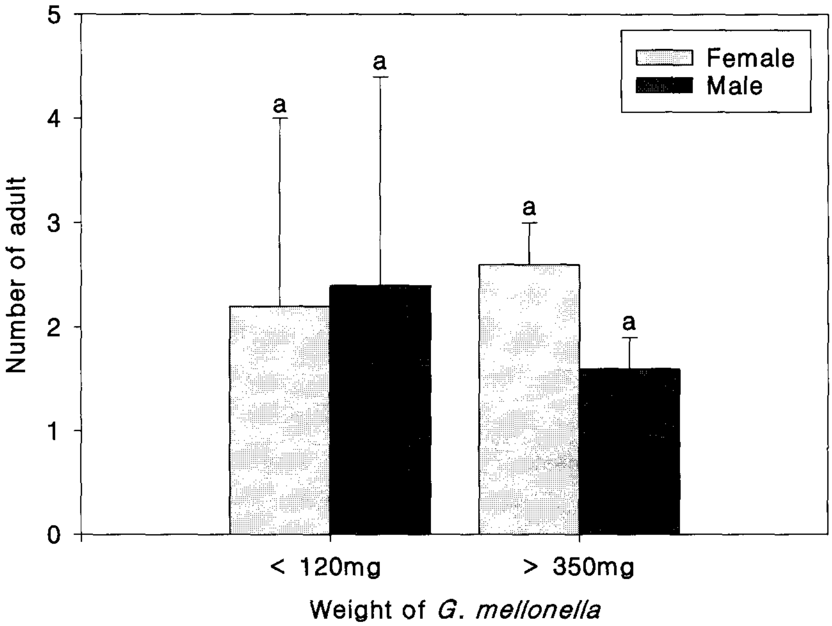

Fig. 1. Effect of host weight on sex ratio of *S. glaseri* NC.

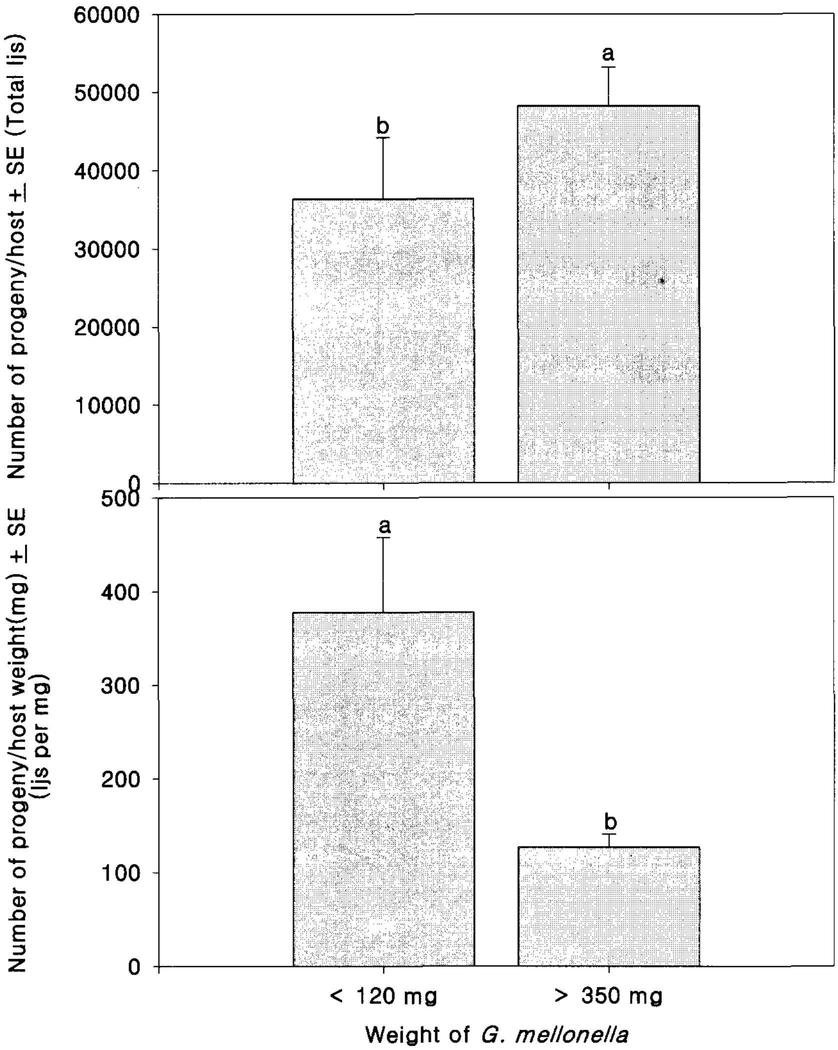

Fig. 2. Effect of host weight on progeny production of *S. glaseri* NC.

48210.0±4930.5 in >350㎎ (T=-1.4; df=15; P<0.05) while that of juveniles per ㎎ was 378.0±80.0 in <120㎎ and 126.8±14.0 in >350㎎ (T=3.7; df=15; P<0.05).

Interrelationship between nematode species and entomopathogenic fungus. The number of females and males matured in the host was variable both in *S. glaseri* NC and in *S. monticola* depending on concentrations. In general, inoculated infective juveniles were matured to be more females than males (T=6.6; df=78; P<0.01) (Table 1 and 2). Total matured adults at the concentrations of over 40 infective juveniles were more than at those of below 20 ones. Temperature influenced the number of adults. Adults of *S. glaseri* NC were more at 30℃ (F=26.1, df=9, 69 in females: F=4.1, df=9, 69 in males; P<0.05) than at 20℃ (F=18.3, df=9, 69 in females; F=4.1, df=9, 69 in males: P<0.05) while those of *S. monticola* were more at 20℃ (F=31.4, df=9, 69 in females; F =9.6, df=9, 69 in males; P<0.05) than at 30℃ (F=8.4, df=9, 69 in females; F=10.9, df =9, 69 in males; P<0.05) (Table 1 and 2). The lowest and highest total number of adults at 20℃ in *S. glaseri* NC were 3.7 and 31.4 at a concentration of 10 and 150 or 250, respectively. At 30℃ the lowest number of adults in *S. glaseri* NC was 5.6 at a concentration of 10 but the highest number of adults 51.2 at that of 200. In the *S.*

Table 1. Number of matured adults depending on concentration and progenies produced from them in *Steinernema glaseri* NC and *S. monticola* at 20℃

Concen -tration	Number of adults ± SE				Number of Ijs ± SE	
	S. glaseri NC		*S. monticola*			
(Ijs)	♀	♂	♀	♂	*S. glaseri* NC	*S. monticola*
10	2.3±0.5d*	1.4±0.4b	3.3±0.6e	1.9±0.4c	20765.0±2783.4a	26371.9±1682.5a
20	5.4±0.7cd	3.0±0.5ab	5.3±0.8de	4.1±0.6c	23471.5±2481.0a	25419.4±1543.7a
40	10.6±0.7cd	5.7±1.3ab	10.9±1.2cd	6.4±1.0bc	26871.6±3399.5a	25003.8±1336.8a
60	14.0±1.0bc	6.6±1.0ab	11.1±1.9cd	5.4±1.1bc	27606.1±1484.1a	24470.0±1007.8a
100	20.3±1.3ab	8.9±1.5a	21.4±1.4ab	11.3±1.0ab	28018.0±1983.2a	27200.4±1326.1a
150	23.4±1.5ab	8.0±1.2a	25.1±1.7ab	11.1±1.5ab	30418.9±2981.5a	26299.8±1968.5a
200	22.4±1.5a	8.7±2.0a	26.6±0.8a	10.3±1.4ab	28379.8±1271.7a	23999.8±1083.4a
250	23.0±2.4a	8.4±1.7a	24.6±2.8ab	13.4±1.9a	27659.0±4617.9a	22638.5±1813.2a
500	19.6±0.5ab	7.0±1.1ab	21.4±1.5ab	7.7±1.2abc	31700.8±3542.8a	27196.3±2826.0a
1,000	16.0±3.3a	4.1±1.1ab	17.7±1.3bc	10.9±1.2ab	5444.3±1964.7b	10856.0±3015.2b

*Means in the colum followed by the same letter are not significantly different (P>0.05).

Table 2. Number of matured adults depending on concentration and progenies produced from them in *steinernema glaseri* NC and *S. monticola* at 30℃

Concen -tration	Number of adults ± SE				Number of Ijs ± SE	
	S. glaseri NC		*S. monticola*			
(Ijs)	♀	♂	♀	♂	*S. glaseri* NC	*S. monticola*
10	4.0±0.9d*	1.6±0.4b	3.4±0.5b	1.7±0.3c	26037.5±1067.1d	19775.0±699.1a
20	10.4±0.8cd	3.3±0.4ab	3.7±1.0b	2.1±0.7c	30562.5±706.0cd	22832.5±2116.4a
40	14.9±2.8cd	4.0±1.0ab	11.0±1.2ab	4.4±0.5bc	34175.0±1619.4bc	20287.5±1352.2a
60	22.1±2.1bc	3.1±0.4ab	10.7±1.1ab	3.0±0.7c	37362.5±1500.1ab	21162.5±1260.8a
100	34.4±3.7ab	5.1±0.8ab	14.6±2.4a	10.9±1.5a	40500.0±1964.2a	26025.0±876.4a
150	33.4±1.7ab	4.6±0.7ab	17.3±2.8a	8.7±1.2ab	35862.5±1227.0abc	22850.0±808.8a
200	42.9±3.0a	8.3±2.0a	16.0±1.6a	8.7±1.1ab	29587.5±1705.3cd	21540.0±706.2a
250	40.9±2.6a	5.1±1.0ab	19.1±1.7a	9.0±0.9ab	30037.5±1523.3cd	23900.0±1907.2a
500	33.6±3.3ab	7.3±1.9a	14.1±1.4a	8.3±0.9ab	25120.0±1988.8d	25025.0±1587.6a
1,000	38.9±3.9a	3.9±0.8ab	10.6±2.9ab	5.9±1.4bc	7387.5±1435.2e	5350.0±1908.6b

*Means in the colum followed by the same letter are not significantly different (P>0.05).

monticola, the lowest number of adults was 5.2 at a concentration of 10 and the highest number of adults 38.0 at that of 250 at 20℃ and 5.1 and 28.1 at those concentrations at 30℃, respectively. However, the number of progenies did not coincide with the number of adults or females. *S. glaseri* NC was produced the most at a concentration of 500 by 31700.8±3542.8 at 20℃ but 100 infective juvenile inoculation was the best at 30℃ by producing 40500.0±1964.2. 27200.4±1326.1 infective juveniles of *S. monticola* were emerged at a concentration of 100 at 20℃ and 26025.0±876.4 at 30℃ (Table 1 and 2). The progeny production was not significantly different at 20℃ ($F=5.8$, $df=9, 79$ in *S. glaseri* NC; $F=5.8$, $df=9, 79$ in *S. monticola*) but variable at 30℃ ($F=32.2$, $df=9, 79$ in *S. glaseri* NC; $F=14.7$, $df=9, 79$ in *S. monticola*). The number of infective juveniles emerged was the lowest at high concentration, 1,000 infective juveniles compared with other concentrations in both species and temperature.

Interaction between nematode species and entomopathogenic fungus. When steinernematids and heterorhabditids were inoculated to a host together in a container, mortality of hosts was generally higher by steinernematid species than by heterorhabditid species at 20℃ ($F=46.5$; $df=11, 35$; $P<0.05$) (Table 3). Mortality was 76.2±4.8 by *S. carpocapsae* Pocheon and 23.8±4.8 by *H. bacteriophora* Hamyang. When *S. carpocapsae* Pocheon and *H. bacteriophora* NC 1 were inoculated together, mortality was 90.5±4.8 by *S. carpocapsae* Pocheon and 9.5±4.8 by *H. bacteriophora* NC 1. The similar trends were shown in the combination of *S. glaseri* NC (61.9±9.5% and 80.9±4.8%) and *H. bacteriophora* Hamyang (38.1±9.5%) or *H. bacteriophora* NC 1 (19.1±4.8%). The number of matured adults and progenies were not different between one species inoculation and two species inoculation ($F=3.7$; $df=23, 111$; $P<0.05$) (Table 4). Mortality ($F=62.0$; $df=11, 35$; $P<0.05$) and number of matured adults ($F=6.1$; $df=23, 111$; $P<0.05$) at 30℃ also showed the same trends as at 20℃ (Table 5 and 6). Simultaneous infection of

Table 3. Infectivity of *Steinernema* and *Heterorhabditis* to *Galleria mellonella* larvae when different nematode species were inoculated together in the same host at 20℃

Nematode	% infected ± SE			
	HbH	HbNC1	ScP	SgNC
HbH	100.0 ± 0.0a*			
HbNC1		100.0 ± 0.0a		
ScP			100.0 ± 0.0a	
SgNC				100.0 ± 0.0a
HbH + ScP	23.8 ± 4.8de		76.2 ± 4.8ab	
HbNC1 + ScP		9.5 ± 4.8e	90.5 ± 4.8a	
HbH + SgNC	38.1 ± 9.5cd			61.9 ± 9.5bc
HbNC1 + SgNC		19.1 ± 4.8de		80.9 ± 4.8ab

*Means in the colum followed by the same letter are not significantly different ($P>0.05$).
HbH and HbNC1, *H. bacteriophora* Hamyang and NC 1 strain; ScP, *S. carpocapsae* Pocheon strain; SgNC, *S. glaseri* NC strain.

Table 4. Number of matured adults when steinernematids and heterorhabditids were inoculated together in the same host at 20℃

Treatment	Number of adult ± SE							
	HbH		HbNC1		ScP		SgNC	
	♀	♂	♀	♂	♀	♂	♀	♂
HbH	1.1±0.3*	0						
HbNC1			0.9±0.3	0				
ScP					1.4±0.2	0.7±0.2		
SgNC							1.7±0.3	1.3±0.3
HbH+ScP	1.5±0.4	0			1.4±0.2	1.2±0.3		
HbNC1+ScP			1.0±0.0	0	1.3±0.2	0.8±0.2		
HbH+SgNC	1.5±0.4	0					1.6±0.4	1.4±0.4
HbNC1+SgNC			1.0±0.0	0			1.5±0.2	1.3±0.4

*Means in the colum followed by the same letter are not significantly different (P>0.05).
HbH and HbNC1, *H. bacteriophora* Hamyang and NC 1 strain; ScP, *S. carpocapsae* Pocheon strain; SgNC, *S. glaseri* NC strain.

Table 5. Infectivity of *Steinernema* and *Heterorhabditis* to *Galleria mellonella* larvae when different nematode species were inoculated together in the same host at 30℃

Treatment	% infected ± SE			
	HbH	HbNC1	ScP	SgNC
HbH	100.0 ± 0.0a*			
HbNC1		100.0 ± 0.0a		
ScP			95.2 ± 4.8ab	
SgNC				100.0 ± 0.0a
HbH + ScP	19.1 ± 4.8d		80.0 ± 4.8ab	
HbNC1 + ScP		23.8 ± 4.8d	76.2 ± 4.8b	
HbH + SgNC	52.4 ± 4.7c			47.6 ± 4.7c
HbNC1 + SgNC		19.1 ± 4.8d		80.9 ± 4.8ab

*Means in the colum followed by the same letter are not significantly different (P>0.05).
HbH and HbNC1, *H. bacteriophora* Hamyang and NC 1 strain; ScP, *S. carpocapsae* Pocheon strain; SgNC, *S. glaseri* NC strain.

both species in a host was not observed.

When entomopathogenic nematodes were simultaneouly inoculated with entomopathogenic fungus to a host, the host was infected only by nematodes. However, co-infection with fungus was observed from 12 hr pre-treatment of *B. brongniartii* with *S. carpocapsae* All and 6 hr pre-treatment of *B. brongniartii* with *H. bacteriophora* NC 1. Mortality was 76.0±5.5% by *S. carpocapsae* All and 24.0±5.7% by *B. brongniartii* in 12 hr Bb+Sc and 36.0±5.1% by *S. carpocapsae* All and 64.0±5.5% by *B. brongniartii* in 24 hr Bb+Sc, respectively (Table 7). The similar trends were also observed between *H. bacteriophora* NC 1 and *B. brongniartii* (Table 8). Nematode, fungus, and nematode-fungus co-infection were observed from 48 hr pre-treatment of *B. brongniartii* in both nematode species. Co-infection significantly influenced progeny production. Infective

Table 6. Number of matured adults when steinernematids and heterorhabditids were inoculated together in the same host at 30℃

Treatment	Number of adult ± SE							
	HbH		HbNC1		ScP		SgNC	
	♀	♂	♀	♂	♀	♂	♀	♂
HbH	1.7±0.3*	0						
HbNC1			1.7±0.3	0				
ScP					1.7±0.4	1.1±0.3		
SgNC							2.9±0.5	1.7±0.3
HbH+ScP	1.0±0.0	0			1.0±0.0	1.0±0.0		
HbNC1+ScP			1.0±0.0	0	1.6±0.2	1.2±0.2		
HbH+SgNC	1.5±0.3	0					3.0±0.5	1.0±0.5
HbNC1+SgNC			1.0±0.0	0			2.2±0.4	1.3±0.4

*Means in the colum followed by the same letter are not significantly different (P>0.05).
HbH and HbNC1, *H. bacteriophora* Hamyang and NC 1 strain; ScP, *S. carpocapsae* Pocheon strain; SgNC, *S. glaseri* NC strain.

Table 7. Interaction between *Beauveria brongniartii* (Bb) and the entomopathogenic nematode, *Steinernema carpocapsae* All (Sc)

Treatment	Concentration / larva	% host mortality		
		Sc	Bb	Sc + Bb
Bb	4.8×10^5	–	100.0 ± 0.0 a	–
Sc	20	100.0 ± 0.0 a	–	–
Bb + Sc	$4.8 \times 10^5 + 20$	100.0 ± 0.0 a	–	–
6Bb + Sc	$4.8 \times 10^5 + 20$	100.0 ± 0.0 a	–	–
12Bb + Sc	$4.8 \times 10^5 + 20$	76.0 ± 5.5 b	–	24.0 ± 5.7 d
24Bb + Sc	$4.8 \times 10^5 + 20$	36.0 ± 5.1 c	–	64.0 ± 5.5 b
48Bb + Sc	$4.8 \times 10^5 + 20$	6.0 ± 1.1 de	16.0 ± 3.4 d	78.0 ± 3.8 a
72Bb + Sc	$4.8 \times 10^5 + 20$	20.0 ± 4.1 d	40.0 ± 7.3 c	40.0 ± 7.1 c
96Bb + Sc	$4.8 \times 10^5 + 20$	4.0 ± 0.5 e	70.0 ± 14.1 b	26.0 ± 6.9 d

Figures followed by different letters in a column are significantly different ($P < 0.05$).

Table 8. Interaction between *Beauveria brongniartii* (Bb) and entomopathogenic nematode, *Heterorhabditis bacteriophora* NC 1 (Hb)

Treatment	Concentration / larva	% host mortality		
		Hb	Bb	Hb + Bb
Bb	4.8×10^5	–	100.0 ± 0.0 a	–
Hb	15	100.0 ± 0.0 a	–	–
Bb + Hb	$4.8 \times 10^5 + 15$	100.0 ± 0.0 a	–	–
6Bb + Hb	$4.8 \times 10^5 + 15$	84.0 ± 8.9 a	–	16.0 ± 8.9 b
12Bb + Hb	$4.8 \times 10^5 + 15$	28.0 ± 5.4 b	–	72.0 ± 19.2 a
24Bb + Hb	$4.8 \times 10^5 + 15$	28.0 ± 4.5 b	–	72.0 ± 4.5 a
48Bb + Hb	$4.8 \times 10^5 + 15$	16.0 ± 5.4 bc	24.0 ± 7.9 c	60.0 ± 12.3 a
72Bb + Hb	$4.8 \times 10^5 + 15$	14.0 ± 4.4 bc	46.0 ± 7.1 c	40.0 ± 5.8 b
96Bb + Hb	$4.8 \times 10^5 + 15$	8.0 ± 2.3 c	70.0 ± 27.4 b	22.0 ± 6.5 b

Figures followed by different letters in a column are significantly different ($P < 0.05$).

juveniles emerged from co-infected cadavers were much fewer than nematode- infected cadavers (Fig. 3 and 4). The number of infective juveniles of *S. carpocapsae* All ranged from 1135.4±906.7 to 5864.4±1776.4 in the co-infected cadavers with *B. brongniartii* and that of infective juveniles of *H. bacteriophora* NC 1 ranged from 2141.4±1373.9 to 4407.4±1415.2 in those with *B. brongniartii*, whereas infective juveniles of *S. carpocapsae* All were 37050.8±2551.7 and those of *H. bacteriophora* NC 1 55220.4±5625.8 in

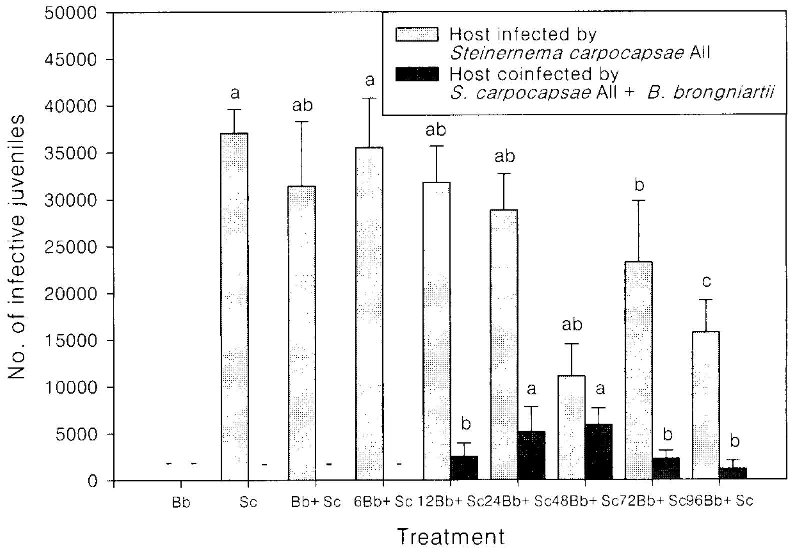

Fig. 3. Number of infective juveniles of *Steinernema carpocapsae* All.

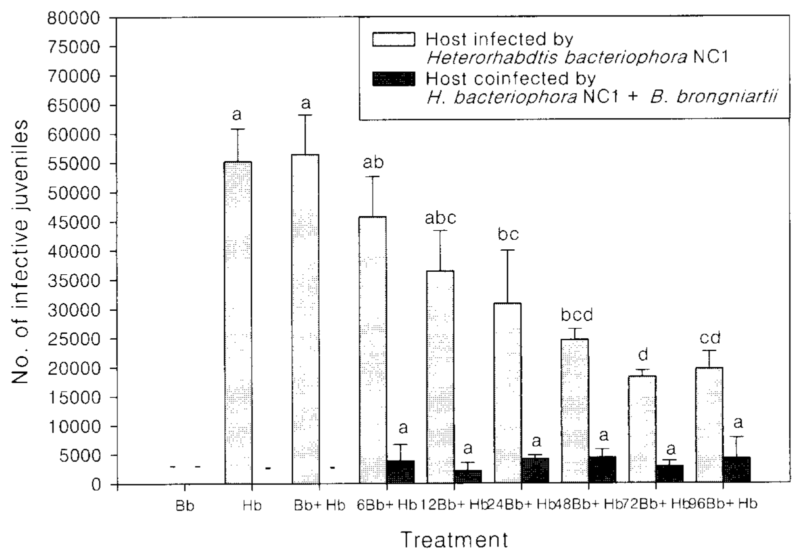

Fig. 4. Number of infective juveniles of *Heterorhabditis bacteriophora* NC1.

nematode-infected cadavers.

DISCUSSION

In general, the last instar larvae of *G. mellonella* were used for biological or ecological experiments or for laboratory propagation of entomopathogenic nematodes. There was little variation resulting from different weight of larvae in some studies (Kondo, 1989; Randy and Campbell, 1989; Selvan *et al.*, 1993). Our results indicated that the weight of larvae should be considered to compare progenies depending on purposes although there was no difference in sex ratios. Woodring and Kaya (1988) suggested about 20 nematodes per larva because too many nematodes per larvae produced few progeny due to competition and/or contamination with foreign bacteria. Density-dependent factors within a host can have an important influence on the population dynamics of parasites. Although the number of infective juveniles that invaded the host increased with increasing dose (Selvan *et al.*, 1993), our results did not coincide with dose. There was no differences in the number of matured adults in a host except low dose, 10 nematodes per host. Although more females were recorded in our experiment, the sex ratios were generally variable according to exposure time or experimental methods and no correlation between the proportion of males and the number of nematodes in cadavers (Lee *et al.*, 1996; Stuart *et al.*, 1998). However, the number of progenies was not different depending on dose or matured adults. The number of progeny was the lowest at a concentration of 1,000 nematodes per host, that is, only 5443.3±1964.7 by 20.1 adults were produced in *S. glaseri* NC at 20℃ and 7387.5±1435.2 by 42.8 adults at 30℃. A lot of progenies were obtaind at other concentrations. High concentrations of nematodes have applied to control pests in many cases but many nematodes were not needed if a certain concentration was enough to kill host. The temperature was also important factor for pathogenicity or nematode production but our results showed no difference between 20℃ and 30℃. Unlike Alatorre-Rosas and Kaya (1991) steinernematids and heterorhabditids were not infected together in the same host. However, the *Galleria* larvae were more infected by steinernematid species than *H. bacteriophora* like Alatorre-Rosas and Kaya's observation (1991). *Steinernema* spp. outcompeted *H. bacteriophora* (Alatorre-Rosas and Kaya, 1990, 1991). Co-inoculation of two different nematode species did not influence progeny production between one species and two species treatments. Our result on intercompetition between steinernematids and heterorhabditids showed the same trend as Alatorre-Rosas and Kaya. About interrelationship between entomopathogenic nematodes and entomopathogenic fungus, application time was important factor. When nematodes and entomopathogenic fungus were inoculated together, insects were mainly infected by nematodes but 48 hr pre-treatment of *B. brongniartii*, *B. bassiana* or *Metarrhizium anisopliae* resulted in high co-infection with higher fungus infection. Infective juveniles were poorly emerged from the co-infected hosts. *S. carpocapsae* or *H. bacteriophora* tended to occur more frequently at the host not infected with *B. bassiana* (Barbercheck and Kaya, 1991). However, *B. bassiana* and nematodes rarely coproduced progeny in dually infected hosts. *B. bassiana* was detrimental to the development of *S. feltiae* and *H. bacteriophora* when applied to the insects more than 48 hr before nematodes (Barbercheck and Kaya, 1990). This was shown from 12 hr pre-treatment of *B. brongniartii* in our experiment. The entomopathogenic fungi obviously inhibited or prevented the development of nematodes. Interrelationship between nematodes and *M. anisopliae* or *B. bassiana* was closely similar to observation on relationship between nematodes and *B. brongniartii* (Choo unpublished data). The hosts infected by fungi might not be suitable for nematodes because host condition was not good enough to produce progenies. Despite progenies of both pathogens were inhibited, combination of these pathogens might resulted in high virulence against pests because simultaneous application of both pathogens shortened period of lethal infection. Recently combined application of nematodes with different species or with other micropathogens was deveoped to control insects effectively (Kaya, 1993; Thurston *et al.*, 1993, 1994; Choo *et al.*, 1996, 1997; Koppenhöfer *et al.*, 1999). This strategy is desirable to enhance nematode efficacy against target insects. Our data supported that a target host or more than two target insects having two different locations even in the same habitat could be controlled by one of applicated nematode species or micropathogens. In addition, stress by a pathogen readily predisposes hosts to nematodes.

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요 약

곤충병원성 선충의 이용 효율을 높이기 위하여 곤충병원성 선충 상호간 또는 선충과 곤충병원성 곰팡이와의 상호관계를 알아 보았다. *Steinernema glaseri*는 기주의 무게에 따라 성충수에서는 차이가 없었으나 침입태 유충수에서는 차이가 있었다. 선충의 접종 농도에 따른 침입 발육된 성충수는 기주당 20마리 이하의 접종에서는 차이를 보였으나 40마리 이상 1,000마리 접종구에서는 차이가 없었고, 증식된 유충수도 기주당 1,000마리 접종구에서 현저히 적은 것을 제외하고는 차이가

없었다. 동일 기주에서의 선충 상호간 관계에서는 steinernematid 선충에 의한 기주 치사율이 heterorhabditid 선충에 의한 것보다 높았다. 즉, 서로 다른 종의 곤충병원성 선충을 동일 기주에 접종하였을 때, *S. carpocapsae* 포천에 의한 기주 치사율은 76.2±4.8%였고 *Heterorhabditis bacteriophora* 함양에 의한 치사율은 23.8±4.8%였다. 또한 *S. carpocapsae* 포천에 의한 기주 치사율이 90.5±4.8%, *H. bacteriophora* NC 1에 의한 것은 9.5±4.8%였다. *S. glaseri* NC와 *H. bacteriophora* 함양 및 *H. bacteriophora* NC 1을 동시 접종하였을 때는 *S. glaseri* NC에 의한 것이 각각 61.9±9.65%와 80.9±4.8%, *H. bacteriophora* 함양에 의한 것이 38.1±9.5%, *H. bacteriophora* NC 1에 의한 것이 19.1±4.8%였다. 그러나 두 선충의 동시 감염은 관찰되지 않았다. 그리고 *S. carpocapsae* All과 곤충병원성 곰팡이인 *Beauveria brongniartii*를 동시 또는 곰팡이를 먼저 처리했을 때는 곰팡이 12시간 전 처리부터 선충과 곰팡이의 동시 감염이 관찰되었고, *H. bacteriophora* NC 1는 곰팡이 6시간 전 처리부터 동시 감염이 관찰되었다. 선충에 의한 감염과, 곰팡이에 의한 감염, 선충과 곰팡이 동시 감염은 곰팡이 48 시간 전 처리부터 관찰되었다. 그러나 유충 증식수는 선충 단독 감염보다 동시 감염충에서 현저히 떨어졌다.

검색어: 상호관계, 침입태유충, *Steinernema carpocapsae*, *S. monticola*, *S. glaseri*, *Heterorhabditis bacteriophora*, *Beauveria brongniartii*

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